Molecular strain characterization in *Wolbachia* phylogeny and evolutionary ecology

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Many questions on *Wolbachia* ecology and evolution can only be addressed through molecular strain characterization. For example, molecular approaches have been employed to detect *Wolbachia* presence, to determine tempo and mode of horizontal *Wolbachia* transfer, or to ascertain deep evolutionary relationships within the genus *Wolbachia*. While each of these tasks demand molecular markers with specific characteristics, most studies fail to address if these characteristics are met.

Here, I will review the use of molecular markers in *Wolbachia* phylogeny, ecology, and strain typing. Specifically, I will discuss the performance of MLST (multi locus sequence typing) in common tasks performed by *Wolbachia* researchers and summarize the state of knowledge on molecular *Wolbachia* phylogeny. I will argue that despite being widely used, MLST markers are problematic for a number of reasons. Therefore, the lack of conclusive patterns that is often observed when investigating *Wolbachia's* evolutionary ecology may well be an artefact of the typically employed markers. On the contrary, although deep-level *Wolbachia* phylogeny appears to be contested, there is very little conflict in genomic datasets.

Finally, using recent examples from the literature, I will argue that only genomic approaches will enable us to understand Wolbachia's evolutionary history.

Mechanisms of establishing symbiosis in *Wolbachia*

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While *Wolbachia* is primarily vertically transmitted via the maternal germline, intraspecific horizontal transfer can occur in nature. Additionally, these intraspecific transfers of *Wolbachia* from one host to another form the basis of many ongoing vector control programs. Establishing *Wolbachia* in a novel host is not trivial. We know relatively little about the requirements for *Wolbachia* to establish a new infection, nor do we understand how *Wolbachia* responds to such a drastic change in environment. Here, we use transcriptomic and molecular approaches to explore how the *Wolbachia* strain *w*Mel responds to moving between host contexts. While *Wolbachia* can survive outside of host cells, we find that once removed from host cells, *Wolbachia* no longer produces essential components of its type IV secretion system (T4SS). *Wolbachia* appear to modulate the expression of their T4SS depending on host contact. Additionally, transcriptomics revealed a suite of other *Wolbachia* loci that are differentially expressed when *Wolbachia* are extracellular versus in association with the host. Results will generate a deeper understanding of how *Wolbachia* initiates a symbiosis with host insects and inform the use of *Wolbachia* as a tool for the control of target pest species.

Symbionts in waiting – the dynamics of incipient endosymbiont complementation and replacement in minimal bacterial communities of insects

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Obligate bacterial primary (P-) endosymbionts that are maternally inherited and codiverge with hosts are widespread across insect lineages with nutritionally restricted diets. Secondary (S-) endosymbionts are mostly facultative but in some hosts they complement P-endosymbiont function and therefore become obligate. Phylogenetic evidence exists for host switching and replacement of S-endosymbionts. We performed bacterial 16S rRNA gene amplicon sequencing of 25 psyllid species (Hemiptera, Psylloidea). All species possessed the P-endosymbiont *Carsonella* (Gammaproteobacteria); most species harboured one dominant representative of diverse gammaproteobacterial S-endosymbionts that was consistently detected across all host individuals and populations (*Arsenophonus* in eight species, *Sodalis* or *Sodalis*-like bacteria in four species, unclassified Enterobacteriaceae spp. in eight species). The identity of this dominant obligate S-endosymbiont varied across closely related host species. Unexpectedly, five psyllid species had two or three co-occurring endosymbiont species other than *Carsonella* within all host individuals, including a *Rickettsiella*-like bacterium (Gammaproteobacteria) in one psyllid species. Some psyllids also had Alphaproteobacteria (*Lariskella*, *Rickettsia*, *Wolbachia*) at varying or fixed prevalence. Our data support the hypothesis of strict vertical transmission of minimal core communities of bacteria in psyllids. We also found evidence for S-endosymbiont replacement across closely related psyllid species. The presence of multiple, dominant S-endosymbionts in some host species constitute potential examples of incipient endosymbiont complementation or replacement. We further tested the potential for metabolic complementation by generating genome sequences of *Carsonella* and its co-occurring S-endosymbionts: *Arsenophonus* (in *Cardiaspina albitextura* and *C. fiscella*), *Sodalis* (in *C. maniformis*) and co-occurring *Arsenophonus* and *Sodalis* (in *Glycaspis sp.*). Comparing the presence/ absence of protein coding genes involved in metabolism in symbiont assemblages across different host species demonstrated complementary functions of primary and secondary symbionts. Our multiple comparisons of deep-sequenced minimal insect bacterial communities exposed the dynamics involved in shaping insect endosymbiosis.

A single prophage WO gene rescues cytoplasmic incompatibility in *Drosophila melanogaster*

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Wolbachia are maternally-inherited, intracellular bacteria at the forefront of vector control efforts to curb arbovirus transmission. In international field trials, the cytoplasmic incompatibility (CI) drive system of *w*Mel *Wolbachia* is deployed to replace target vector populations, whereby a *Wolbachia*–induced modification of the sperm genome kills embryos. However, *Wolbachia* in the embryo rescue the sperm genome impairment, and therefore CI results in a strong fitness advantage for infected females that transmit the bacteria to offspring. The two genes responsible for the *w*Mel-induced sperm modification of CI, *cifA* and *cifB*, were recently identified in the eukaryotic association module of prophage WO, but the genetic basis of rescue is unresolved. Here we use transgenic and cytological approaches to demonstrate that *cifA* independently rescues CI and nullifies embryonic death caused by *w*Mel *Wolbachia* in *Drosophila melanogaster*. Discovery of *cifA* as the rescue gene and previously one of two CI induction genes establishes a new 'Two-by-One' model that underpins the genetic basis of CI. Results highlight the central role of prophage WO in shaping *Wolbachia* phenotypes that are significant to arthropod evolution and vector control.

name names: substrates and suppressors of CidB, the CI inducing deubiquitylase.

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Transgenic cytoplasmic incompatibility (CI) can be induced by a deubiquitylating enzyme, CidB. Our studies present genetic and proteomic results identifying conserved physical and genetic interactions with CidB in both *Saccharomyces cerevisiae* and *Drosophila melanogaster.* CidB induces a toxic phenotype in yeast. We screened a high copy yeast genomic tiling library to identify suppressors of CidB toxicity. Three strong and highly reproducible suppressors were identified. Yeast genetic analyses were followed by substrate trapping pulldowns of recombinant epitope tagged CidB with *Drosophila melanogaster* protein extracts. We produced three triplicate mass spectrometry of proteomes defining proteins uniquely enriched in the presence of CidB, in the presence of CidA, and in the presence of both CidA and CidB together. All three proteomes highlight mutually exclusive interactions suggesting that substrates of the CidB enzyme cannot be bound when in the presence of CidA. These data support a hypothesis where CidA rescues CI by altering localization and substrate specificity of the key enzyme CidB. Notably, our strongest yeast suppressor was also our most robust physical interaction identified in *Drosophila*. Thus, our data supports a conservation of basic biochemical mechanisms across yeast and fruit flies and brings the field closer to a definitive mechanism for CI induction and rescue.

Toxin-Antitoxin Modules Drive Cytoplasmic Incompatibility by Intracellular *Wolbachia* Bacteria

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Wolbachia are obligate intracellular bacteria that infect millions of species, including some two-thirds of all insect species. These symbionts manipulate host reproduction to enhance their inheritance through the female germline. The most common mechanism of reproductive alteration is called cytoplasmic incompatibility (CI), wherein eggs from uninfected females fail to develop when fertilized by sperm from *Wolbachia*-infected males, but embryos are fully viable if infected females mate with either infected or uninfected males. CI is a potent gene-drive mechanism that impacts population structure and evolution of new species, but its molecular mechanisms have remained unclear. We show that *Wolbachia* operons encoding either a deubiquitylating enzyme (DUB) called CidB or a nuclease called CinB can induce CI. In transgenic fruit flies, the DUB or nuclease appears to act as a toxin when sperm introduce either into eggs. The *Wolbachia* DUB and nuclease proteins are encoded in two-gene operons in which the upstream genes encode factors that bind in a cognate-specific fashion to these enzymes. When expressed in yeast, the DUB or nuclease proteins are also toxic, and their enzyme activity is required for toxicity. Toxicity is suppressed by co-expression of the cognate upstream factor (CidA or CinA). Our data suggest distinct mechanisms for CI involving toxin and antitoxin-like proteins secreted into germline cells by resident bacteria. These results may have practical applications in limiting disease vectors, such as the mosquitoes that carry Zika and dengue fever viruses, or crop pests.

What is the role of phage WO in cytoplasmic incompatibility?

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Wolbachia confers insect resistance to a number of RNA viruses and encodes a natural drive system that allows it to spread through mosquito populations, a process known as cytoplasmic incompatibility (CI). Two genes in the eukaryotic association module of prophage WO of *Wolbachia* – *cifA* and *cifB* – can recapitulate CI. However, the role of phage WO particles in the induction of CI remains unclear. Prophage WO actively produces viral particles in the *w*VitA *Wolbachia* strain infecting *Nasonia vitripennis*, but it is unknown whether the phages play a role in CI. To test the hypothesis that phage WO particles transport the Cif proteins, we injected uninfected male *Nasonia* with PEG precipitated protein containing WO VitA particles, which resulted in recapitulation of the CI phenotype. Additionally, we confirm that prophage WO induces viral particles in the released *Wolbachia* strain used for vector control (*w*Mel) within *Drosophila melanogaster*. These viral particles appear structurally similar to previously described WO particles from other strains and occur both inside and outside the *Wolbachia* cells inhabiting the testes. Phage injection experiments were replicated in *Drosophila* using the *w*Mel strain, reiterating results observed in *Nasonia*. DNA staining highlights localization patterns of injected phages in the reproductive tissues. This emerging project on the mechanistic basis of CI aims to (i) provide insight into the functions of phage WO, (ii) determine how the Cif proteins are transported to the sperm to confer CI, and (iii) inform ongoing and future vector control efforts enabled by *Wolbachia* and prophage WO genes.

Wolbachia stimulate the egg production and are essential to the germline stem cell homeostasis

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The presence of *Wolbachia* in the female germline is essential in filarial species to ensure a proper *in utero* embryonic development. After depletion of the Wbm symbionts from the filarial nematode *Brugia malayi*, a massive apoptosis occurs in embryos. We however detected developmental defects in early embryos prior to apoptosis, suggesting that the primary role of *Wolbachia* is not to prevent apoptosis, but rather to contribute to the proper embryonic cell fates and tissue differentiation. In order to look for the earliest defects triggered by the loss of *Wolbachia*, we applied newly developed techniques to demonstrate the requirements of *Wolbachia* in the parasite germline preceding the production of faulty embryos. We show that *Wolbachia* stimulate germline proliferation in a cell-autonomous manner, and found *Wolbachia* to maintain the quiescence of a pool of germline stem cells to ensure a constant delivery of about 1400 eggs per day for many years. The loss of quiescence upon *Wolbachia* depletion, as well as the disorganization of the distal germline suggest that *Wolbachia* are required to execute the proper germline stem cell developmental program in order to produce viable eggs and embryos.

How *Wolbachia* survive in eukaryotic cells? Focus on host defense and metabolic processes altered by bacteria.

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Interactions between intracellular bacteria and eukaryotic cells are essential for bacterial survival and replication. Endosymbiotic bacteria *Wolbachia* have shown an impressive wide range of symbiotic association with its host (ranging from reproductive parasitism in arthropods, to mutualistic relationships observed in wasps and parasitic filarial nematodes). Our research is focused on how intracellular bacteria can survive in eukaryotic cells. In particular, we study the ability of *Wolbachia* to escape from intracellular defense, participating in host metabolic processes in favor to obtain nutrition such as glycolytic metabolites, fatty acids and amino acids from cytoplasm of insect cells and human filarial parasites. Finally, we will discuss the role of host miRNAs that are regulated by the bacteria in order to manipulate protein synthesis in filarial nematodes. Specifically, we will discuss outcomes that can be translated from understanding the mechanisms of bacteria-host interactions to drug target discovery projects.

Evolution and functional characterization of Wolbachia type IV secretion substrates

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Wolbachia pipientis is an intracellular symbiont of arthropods well known for the reproductive manipulations induced in the host and, more recently, for the ability of *Wolbachia* to block virus replication in insect vectors. Since *Wolbachia* cannot yet be genetically manipulated, and due to the constraints imposed when working with an intracellular symbiont, little is known about mechanisms used by *Wolbachia* for host interaction. Here we discuss our progress in understanding the mechanisms by which *Wolbachia* manipulate host biology. Specifically, we focus on the type IV secretion system and its substrates. We first employed a bioinformatics pipeline and identified 163 candidate effectors, potentially secreted by *Wolbachia* into the host cell. A total of 84 of these candidates were then subjected to a screen of growth defects induced in yeast upon heterologous expression which identified 14 top candidates likely secreted by *Wolbachia*. These predicted secreted effectors may function in concert as we find that their native expression is correlated during *Drosophila* development as are their evolutionary histories. Similarly, most of these predicted effectors, identified in strain *w*Mel, are limited to one or two *Wolbachia* clades – perhaps reflecting shared evolutionary history and strain specific functions in host manipulation. For one of these effectors, WalE1, we used biochemistry and heterologous assays to identify its function as an actin bundling protein. We go on to describe a heterologous secretion assay for these effectors, based on the *E. coli* conjugatory pilus, and confirm interaction between *Wolbachia* T4SS components WalE1. Finally, we identify host targets of WalE1 through native coimmunoprecipitation. Identification of these *Wolbachia* candidate effectors is the first step in dissecting the mechanisms of symbiont-host interaction in this important system.

Impact of the *Wolbachia* endosymbiont on the AGH pathway in the common woodlouse (*Armadillidium vulgare*)

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Male sex differentiation in malacostracan crustaceans is determined by the presence of the Androgenic Gland Hormone (AGH), a peptidic hormone of the insulin superfamily. In isopods, this hormone is only expressed in genetic males. In populations infected by feminizing *Wolbachia* strains, when male embryos inherit the bacteria, the AGH is not expressed during development and genetic males develop into fully functional females, leading to female-biased sex-ratios. The AGH signalling pathway is thus a putative target of the bacteria but to this date, the mechanisms underlying this endocrine disruption remain unknown. Although the AGH has been studied for decades, its first molecular partners have been discovered and described only recently, in decapod crustaceans: a carrier called Insulin-like Growth Factor Binding Protein (IGFBP) and the membrane receptors called Insulin-like Receptors (IRs). We identified the homolog proteins in isopods and characterized them in our model species: the common woodlouse (*Armadillidium vulgare*). We investigated in particular their structure, their expression during development and their spatial expression in adults, comparing both sexes in naturally *Wolbachia*-infected and uninfected lineages. *Wolbachia* transinfection and RNAi experiments allowed further investigation of the AGH receptor implication in the feminization process.

Tempo and Mode of *Wolbachia* Acquisition across *Drosophila*

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We review recent results concerning *Wolbachia* found in *Drosophila* species, with an emphasis on how and when they were acquired and whether they alter host reproduction. In a pioneering survey of 1225 strains from 225 *Drosophila* species then available from the *Drosophila* species stock center, Mateos *et al*. (2006, Genetics 174:363–376) found 19 *Wolbachia*-infected species. They did not determine the effects of newly discovered infections, but the *Wolbachia* were characterized with the respect to 16S and *wsp*. The Hoffmann, Cooper and Turelli labs have assayed about 60 *Drosophila* species. We found 27 *Wolbachia*-infected species, including 24 from the roughly 191-member *melanogaster* species group. For 25 of these infections, we have draft *Wolbachia* genomes. For all infected species for which uninfected stocks could be generated, we assayed reproductive effects. We found that most *Wolbachia* infections maintained in laboratory stocks cause cytoplasmic incompatibility. However, the *Wolbachia* infections in the *montium* subgroup species *D. barbarae* and *D. nikananu* cause no detectable reproductive effects, like *w*Au in *D. simulans* and the *Wolbachia* infections in *D. mauritiana*, *D. suzukii* and *D. subpulchrella*. Examining host nuclear genes, mitochondrial genomes and *Wolbachia* genomes, we have found no instances of cladogenic *Wolbachia* transmission within the *melanogaster* species group. Instead when very closely related hosts carry very closely related *Wolbachia*, acquisition often occurs through hybridization and introgression. We have several instances of interspecific horizontal acquisition, but analyses of mitochondrial and *Wolbachia* genomes suggest that intraspecific horizontal transmission and paternal transmission are extremely rare. Plausible calibrations of rates of *Wolbachia* divergence relative to mitochondrial and nuclear divergence suggest that many *Wolbachia* infections have been recently acquired, within tens of thousands of years or less. This suggests that *Wolbachia* strain replacement, as observed in eastern Australian *D. simulans* (Kriesner *et al*. 2014, PLoS Pathogens 9:e1003607) may be relatively common.

Comparative population genomics demonstrates introgressive and horizontal transfer of *Wolbachia* among hybridizing *Drosophila yakuba*-clade hosts

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The three *Drosophila yakuba*-clade species ((*D. santomea, D. yakuba*), *D. teissieri*), within the nine-species *Drosophila melanogaster* subgroup, diverged about three million years ago and currently hybridize in west Africa on the islands of Bioko and São Tomé. Each species is polymorphic for very similar *Wolbachia* infections that are closely related to *w*Mel, globally polymorphic in *D. melanogaster*. These *Wolbachia* cause weak cytoplasmic incompatibility that depends on both host and *Wolbachia* genetic variation, and *Wolbachia* infection frequencies vary spatially and temporally in west Africa. As with most host species, the predominant mode(s) by which *D. yakuba*-clade species acquire *Wolbachia* remains uncertain. Species-specific associations could be old, with *Wolbachia* co-diversifying with their hosts (i.e., cladogenic transfer), or relatively young and acquired by either non-sexual horizontal transfer or via interspecific hybridization and introgression (possibly including paternal transmission). In principle, all three modes of *Wolbachia* acquisition are possible in the *D. yakuba* clade. We use draft *Wolbachia* and mitochondria genomes to demonstrate that *Wolbachia* and mitochondrial phylogenies tend to follow that of their hosts; however, several *D. santomea* individuals, sampled from both inside and outside of the São Tomé hybrid zone, have introgressed *D. yakuba* mitochondria. Both mitochondria and *Wolbachia* possess much more recent common ancestors than the bulk of the host nuclear genomes, supporting introgression and precluding cladogenic *Wolbachia* acquisition. Rare examples of discordance of mitochondrial and *Wolbachia* phylogenies indicate rare horizontal or paternal *Wolbachia* transmission within species. Taken together, our analyses provide a comprehensive view of *Wolbachia* movement through a clade of hosts with multiple contemporary hybrid zones.

Developing the true bug-*Burkholderia* symbiosis as a model for studying environmental symbiont acquisition

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Many members of animal-associated microbial communities, including the skin and gut flora, are acquired from their host's environment. Acquisition of free-living symbionts presents a unique set of potential challenges and rewards for a host. Risks include potential failure to find the symbiont and increased chances of the symbiont becoming pathogenic. However, free-living microbes directly interact with, and adapt to, their local environment. Acquisition of the right strain could therefore supply a host with instant adaptation to local conditions and could even promote niche expansion to new diets or climates.

We are developing hemipteran bugs (the stilt bug *Jalysus wickhami* and the leaf-footed bug *Leptoglossus zonatus*) and their *Burkholderia* bacterial symbionts as a system to investigate the costs and benefits of environmental symbiont acquisition. These bugs host *Burkholderia* in sac-like outgrowths called "crypts" at the end of the midgut. Young nymphs acquire their symbionts from the environment every generation. Individual insects commonly host 1 to 3 strains of *Burkholderia*, and across individuals the overall diversity hosted is greater than 60 strains. Experimental elimination of symbionts causes retarded growth, increased mortality, and reduced reproductive output for host insects.

We will assess whether symbiotic *Burkholderia* help their host cope with environmental stressors that vary in space or time, such as dietary toxins, pathogens, the nutritional quality of the host's diet, and temperature. This work will address the unique potential for acquired free-living symbionts to adapt to and confer host tolerance to the specific conditions of a host's local environment.

*w*Ana Genome from MinION Long-Read Sequencing Data: DNA Preparation to Assembly

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Increasingly accessible and economic sequencing enables genome sequencing projects in laboratories outside of dedicated genome facilities. Specifically, Oxford Nanopore Technologies MinION sequencer has a low upfront cost ($1000 USD); a footprint the size of a harmonica; runs on a personal computer; and can reliably generate 15 Kbp reads with no theoretical upper limit on read length. Despite their higher error rates, these longer reads have important applications in *de novo* assembly, structural variations, and elucidation of complex genomic regions. We present the complete genome of *Wolbachia* strain "*w*Ana" that was sequenced directly from *Drosophila ananassae*. The 12-hour sequencing run was done on a single flow cell for $1000 USD with 10 µg of DNA using a rapid, 30-minute library prep that generated 107,811 reads totaling 1.07 Gbp with a read N50 of 18.6 Kbp and a longest read of 171 Kbp. The assembly contains a single 1.33 Mbp contig constructed from just 2,694 reads totaling 22.4 Mbp with a longest read of 114 Kbp. The *w*Ana genome is very similar in content and synteny with *w*Ri, containing just a few inversions and one major deletion. The ultra-long reads were able to very clearly and easily elucidate the two nearly identical >40 kbp prophage and reveals that one of the prophage is missing a large section present in the other. We will compare the MinION assembly to previous data generated using the Roche 454 and Illumina platforms in order to address the advantages and disadvantages this platform provides for sequencing endosymbiont genomes.

Large Fragment Targeted Enrichment Capture of *Wolbachia* genomes to diversity study: Supergroup J *Wolbachia* is not yet buried.

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Although most filarial nematodes (Nematode: Onchocercidae) infecting humans contain the endosymbiotic alpha-proteobacteria *Wolbachia*, only approximately half of all studied filariae (46 on 87 studied species) harbor this symbiont. The symbiosis between filarial nematodes and *Wolbachia* is considered to be mutualistic. Indeed, it has been demonstrated that antibiotic treatments lead to a decreased fertility of adult worms and they can even be macrofilaridal. However, symbiotic mechanisms remain unclear and have largely been inferred from genomic analysis. Additional genomic data for a variety of *Wolbachia* clades might help to characterize the nature of the symbiosis. One main challenge of the *de novo* assembly of *Wolbachia* is the presence of host DNA (both nematode and vertebrate host) in the sample. To get rid of this "Russian nested dolls" effect, we are using an approach based on biotinylated probes to capture large fragments of *Wolbachia* DNA for PacBio sequencing. We have used this approach to capture and sequence *Wolbachia* from several clades and have successfully captured 2-3 kb *Wolbachia* fragments from *Brugia malayi* filarial nematode (supergroup D *Wolbachia*) as a known sequence control to validate the method and from *Cutifilaria tuberocauda* filarial nematode (supergroup J *Wolbachia*). From *B. malayi*, PacBio sequencing of the captured DNA results of an enrichment of *Wolbachia* DNA by 28-fold, from 2.6% of the reads to 56% of the reads. The sequenced reads enable *de novo* assembly of a circularized contig of 1,080,055bp, 99.996% identical to the *wBm* reference with 18 SNPs confirmed by PCR. For *C. tuberocauda*, we processed captured PacBio libraries and an Illumina library without the capture method. We produced a polished circularized *Wolbachia* genome of 863,988 bp. This *wCtub* genome is the first representative of supergroup J clade and the smallest of known *Wolbachia* genomes from filarial nematodes.

Wolbachia Lateral Gene Transfer Events in Insects: A Whole Genome Analysis of Bugs and Beetles

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Lateral gene transfer (LGT) has been recognized for decades within the bacterial domain of life. Inter-domain LGTs are being increasingly described from prokaryotes to eukaryotes, and in particular, from *Wolbachia* to insect genomes such as in *Callosobruchus chinensis* and *Drosophila ananassae*. Given the prevailing estimate that nearly half of insects are carriers of *Wolbachia*, there are likely more *Wolbachia*-to-insect LGTs to be found. To determine how widespread *Wolbachia* LGT events might be in insects, we used a robust bioinformatics pipeline, LGTSeek, to compare the *Wolbachia* donor reference sequences to the raw sequence reads of potential recipient insects. The Sequence Read Archive (SRA) for publicly available DNA sequences contains >21,000 biosamples with raw whole genome sequencing (WGS) data from representing 13 of the 26 insect orders. We have focused our early analysis on two insect orders, Coleoptera and Hemiptera, that offer taxonomic diversity, contain at least one insect species known to be a *Wolbachia* carrier, and present a manageable number of genomes. There are 105 Coleoptera raw WGS datasets from 11 insect families and 33 insect species, while there are 215 Hemiptera raw WGS from 14 families and 20 species. We detected *Wolbachia* reads in 23% of the genomes already run through the pipeline and potential LGTs in 70% of these. Of the potential LGTs, the western corn rootworm (*Diabrotica virgifera*) generated by Monsanto contain, on average, 82 reads per sample that strongly support the presence of at least one LGT from a *Wolbachia* endosymbiont genome to the *Diabrotica virgifera* genome. This result, and others, will be discussed further.

Multiple Lateral Genetic Transfers between *Wolbachia pipientis* and *Aedes albopictus*

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The endosymbiont *Wolbachia* is present in the majority of insect species worldwide, including mosquitoes such the dengue and chikungunya vector *Aedes albopictus.* It is clear that there is genetic exchange between *Wolbachia* and host genomes, but the frequency of these exchanges, and their biological impact, still needs to be assessed in the majority of insect hosts. The size of the inserting genetic material ranges from small fragments (<1000 bp), or entire genomes, into the nuclear DNA of its host species. Here we demonstrate that the wAlbA/wAlbB *Wolbachia* superinfection present in *Aedes albopictus* has resulted in frequent modification events in the mosquito genome, with over 100 detected instances of *Wolbachia* lateral DNA transfer identified in the genomes of four geographically distinct *Aedes albopictus* strains (Kuala Lumpur, Hawaii, La Reunion and Malaysia). The majority of these transfer events originate from the *Wolbachia* strains currently infecting *Aedes albopictus*, wAlbA and wAlbB, though we also identify some gene transfer fragments may originate from an additional *Wolbachia* strain no longer present in *Aedes albopictus.* These findings are important as we start to understand and use artificial *Wolbachia* infections in mosquitoes for vector control purposes.

RNAs as potential targets for *Wolbachia*-mediated phenomena

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Wolbachia, endosymbiotic bacteria prevalent in invertebrates, manipulate their hosts in a variety of ways; they induce cytoplasmic incompatibility, male lethality, male-to-female transformation and parthenogenesis. We revealed that, in *Drosophila melanogaster, Wolbachia* infection restored defective *Sex-lethal* (*Sxl*) mutant female germline stem cells (GSCs) through the *Wolbachia* effector protein TOxic Manipulator of Oogenesis (TomO). TomO targeted host *nanos* mRNAs and hindered their interaction with a translational repressor Cup, a component of the maternal ribonucleoprotein (RNP) complex. The resulting enhancement of Nanos prevented the premature differentiation of GSCs, the discernible defects caused by the *Sxl* mutation.

Another fascinating feature of *Wolbachia* is their ability to induce positive-stranded RNA virus resistance in host cells. The histochemical and biochemical analyses revealed that *Wolbachia* closely associate with Dengue virus genomic RNAs and hamper amplification of Dengue single-round infectious particles, which indicate that replication of viruses could be prohibited by *Wolbachia*. The *Drosophila* maternal RNP complexes associated with *Wolbachia* are reported to contain various RNA binding proteins, some of which are the components of the RNA virus replication machinery. We are now testing the hypothesis that the *Wolbachia*-RNP interaction is also the causal element of the RNA virus blocking by *Wolbachia*.

The third Male Killer: Characterization of Male-killing *Wolbachia* in oriental tea tortrix *Homona magnanima*

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Some endosymbionts are known to cause sex-ratio distortion via feminization, parthenogenesis and male-killing. Two male-killing agents, presumed RNA virus (MKV) and *Spiroplasma* have been identified from the oriental tea tortrix *Homona magnanima* (Tortricidae, Lepidoptera) in Japan. Although three *Wolbachia* strains (*w*Hm-a, -b and -c) have also been identified from Japanese populations of *H. magnanima*, none of them showed male-killing as their phenotype. We recently found a new *Wolbachia* strain, *w*Hm-t, caused sex-ratio distortion in Taiwanese populations of *H. magnanima*. Here, we characterized *w*Hm-t in terms of phylogeny, density, and prevalence in field populations to elucidate the difference in phenotypes of four *Wolbachia* strains. From phylogenetic analysis using *wsp* and MLST genes, non-male-killing strain *w*Hm-c and male-killing strain *w*Hm-t showed 100% similarity. The density of *w*Hm-t was significantly higher than that of any other *Wolbachia* strains, and 100-fold difference in titer was observed between *w*Hm-t and *w*Hm-c. The density of *Wolbachia* also differed between two *w*Hm-t infected host lines: 'W^{TH}' (High-density line) and 'W^{TL}' (Low-density line). In W^{TH} line, almost all individuals were killed during embryonic stage with high mortality (> 80%), whereas only males were killed during both embryonic and larval stage in W^{TL} line. The male larvae of W^{TL} line exhibited white body color, which was similar to the symptom of larvae infected with MKV. The crossing experiments between Japanese and Taiwanese *H. magnanima* showed that host genetic backgrounds did not affect *Wolbachia* phenotypes. We then conducted a field survey in Taiwan and found that over 90% of collected egg-masses were positive for *w*Hm-t. However, male-killing was only observed in nearly 50% of egg-masses, which were infected with high *w*Hm-t density. Given these results, male-killing in *H.magnanima* caused by *Wolbachia* is likely to depend on *Wolbachia* strain and its density.

Late male killing caused by novel RNA viruses Partitiviridae in a tea pest, *Homona magnanima*

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Male killing, which results in the biasing of host sex ratio in favor of females, is divided into two types, early and late, and early male killing has evolved in phylogenetically diverse bacteria, including *Wolbachia*, *Rickettsia*, *Arsenophonus*, *Spiroplasma*, and *Flavobacterium*. On the other hand, late male killing has been reported notably in two cases, such as Microsporidia. We have previously reported that a late male-killing agent in *Homona magnanima*, which contained two unique RNA fragments of MK1068 and MK1241 (male-killing virus: MKV). Recently, we found that MKV contained three sequences encoding partitivural RNA-dependent RNA polymerase (RdRp), one sequence MK1068 encoding capsid protein (CP), and one function-unknown MK1241. To clarify whether MKV infection status affects male-killing phenotype, we firstly inoculated purified viral particles into the larvae of the normal sex ratio (NSR) strain reared in the laboratory. The RT-PCR analysis using the MKV specific primers showed that infection of all MKVs in adult females was necessary for female-biased sex ratios in their offspring. In addition, a ten-fold serial dilution inoculation showed that late male killing occurred dose-dependently. We also surveyed the MKV prevalence in field-collected *H. magnanima* and its sex ratio in the next generation. The MKV prevalence was 42.1-59.5% in February and 42.1-89.5% in November in 2017 and MKVs co-infection was observed in both females and males of field-collected *H. magnanima*. The sex ratios were biased in female adults harboring all MKVs; however, for one adult without MK1068 segment showing a significant female-biased sex ratio (F:M = 53:17). Our results suggested that at least four partitiviral segments should infect adult females of *H. magnanima*, and the infection density was also responsible to a biased sex ratio.

A maternally transmitted male killer in *Drosophila biauraria*: Partitivirus as a reproductive manipulator of insects?

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A maternally inherited, all-female trait is widely found among arthropods, which is caused by bacterial endosymbionts of diverse taxa. We discovered a maternally transmitted all-female trait that is not associated with bacteria in *Drosophila biauraria*. The all-female trait is transmissible to normal lines by microinjection. The egg hatch rates suggested early male killing as the mechanism of the all-female trait. Ribosomal RNA-depleted RNA-Seq and fluorescence *in situ* hybridization (FISH) experiments suggested that Partitivirus, a double-stranded RNA virus well described in plants and fungi, residing in cytoplasm of *D. biauraria* is the causal agent of male killing. Recent accumulation of NGS data suggested that Partitivirus is likely to be a vertically transmitted endosymbiont of diverse insect species. We propose that Partitivirus is a new member, and a first member as a virus, of reproductive manipulators of insects.

Male-killing toxin produced by a bacterial symbiont

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Insect symbioses, in which microorganisms are living together with insect hosts, are one of the most extreme examples of host-microbe interaction. Several endosymbiotic bacteria, like *Wolbachia*, *Spiroplasma*, and *Rickettsia* manipulate host reproduction in a selfish way to efficiently spread their infection into a population. *Spiroplasma poulsonii* (hereafter *Spiroplasma*) is a helical-shaped and motile, Gram-positive bacterium, which resides in diverse *Drosophila* species. The remarkable feature of this *Spiroplasma* strain is male killing, whereby male offspring from infected female hosts are selectively killed during development. Although male killing caused by *Spiroplasma* has been described as early as the 1950s, the underlying mechanism, especially the bacterial responsible factor has been elusive. In this presentation, I will focus on a newly identified *Spiroplasma* protein whose expression recapitulates pathology and cell biological phenotypes observed in *Spiroplasma*-infected male embryos of *D. melanogaster*. This *Spiroplasma* protein contains ankyrin repeats and a deubiquitinase domain, which are required for its subcellular localization and toxicity. Our study highlights the existence of a bacterial protein that affects host cellular machinery in a sex-specific manner, which will provide novel insights into both fundamental and applied entomology.

wmk is a *Wolbachia* prophage gene that kills male *Drosophila*

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Despite significant impacts of *Wolbachia* on animal reproduction, evolution, and vector control, the microbial genes underlying most of *Wolbachia*'s reproductive manipulations remain elusive. Here, we demonstrate the discovery of a single gene in the eukaryotic association module of *Wolbachia*'s prophage WO that kills male *Drosophila* embryos. The gene, hereafter denoted *WO male killing* (*wmk*), is located near cytoplasmic incompatibility genes *cifA* and *cifB* and putatively encodes two XRE-family, helix-turn-helix domains. It causes male lethality when transgenically expressed in uninfected *Drosophila melanogaster*. Specifically, *wmk* transgene expression results in a female-biased sex ratio, reduced hatching of male embryos, several male-biased cytological defects during early embryonic development that are typical of *Wolbachia*-induced male killing, and altered transcription across the fly genome. The discovery of *wmk* advances genetic studies of microbial-induced male killing. It also highlights the significance of prophage WO genes in shaping selfish symbiont phenotypes and informs their potential in suppression or modification of pest and vector populations.

Incidence of bacterial endosymbionts in spider mites associated with local environments and host plants

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Spider mites are frequently associated with multiple endosymbionts whose infection patterns often exhibit spatial and temporal variation. However, the association between endosymbiont prevalence and environmental factors remains unclear. Here, we surveyed endosymbionts in natural populations of the spider mite, *Tetranychus truncatus*, in China, screening 935 spider mites from 21 localities and 12 host plant species. Three facultative endosymbiont lineages, *Wolbachia*, *Cardinium*, and *Spiroplasma*, were detected at different infection frequencies (52.5%, 26.3%, and 8.6%, respectively). Multiple endosymbiont infections were observed in most local populations, and the incidence of individuals with the *Wolbachia*-*Spiroplasma* coinfection was higher than expected from the frequency of each infection within a population. Endosymbiont infection frequencies exhibited associations with environmental factors: *Wolbachia* infection rates increased at localities with higher annual mean temperatures, while *Cardinium* and *Spiroplasma* infection rates increased at localities from higher altitudes. *Wolbachia* was more common in mites from *Lycopersicon esculentum* and *Glycine max* compared to those from *Zea mays*.

The bacterial diversity and community composition of spider mites fed five host plants after communities were modified following tetracycline exposure were also investigated. Bacterial diversity tended to decrease after antibiotic exposure but was also influenced by host plant type. In particular, abundance of the maternally inherited endosymbionts *Wolbachia* and *Spiroplasma* was reduced following antibiotic exposure although the extent of reduction differed among host plants. There was an overall tendency for daily fecundity to be lower in the mites with reduced bacterial diversity following the antibiotic treatment.

This study highlights that host-endosymbiont interactions may be associated with environmental factors, including climate and other geographically linked factors, as well as the host's food plant. Our data also suggest that host plants and antibiotics can shape spider mite bacterial communities and that bacterial symbionts improve mite performance.

Multi-locus sequence typing of the sheep tick *Ixodes ricinus* and its symbiont *Candidatus* Midichloria mitochondrii across Europe reveals evidence of co-cladogenesis in Scotland

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Ticks have complex microbiomes, but only a small proportion of the bacterial symbionts recorded from ticks are vertically transmitted. Moreover, co-cladogenesis between ticks and their symbionts, indicating an intimate relationship over evolutionary history driven by a mutualistic association, is the exception rather than the rule. One of the most widespread tick symbionts is *Candidatus* Midichloria, which has been detected in all of the major tick genera of medical and veterinary importance. In some species of *Ixodes*, such as the sheep tick *Ixodes ricinus* (infected with *Ca*. Midichloria mitochondrii), the symbiont is fixed in wild adult female ticks, suggesting an obligate mutualism. However, almost no information is available on genetic variation in *Ca*. M. mitochondrii or possible co-cladogenesis with its host across its geographic range. Here, we report the first survey of *Ca*. M. mitochondrii in *I. ricinus* in Great Britain and a multi-locus sequence typing (MLST) analysis of tick and symbiont between British ticks and those collected in continental Europe. We show that while the prevalence of the symbiont in nymphs collected in England is similar to that reported from the continent, a higher prevalence in nymphs and adult males is apparent in Wales. In general, *Ca*. M. mitochondrii exhibits very low levels of sequence diversity, although a consistent signal of host-symbiont co-cladogenesis was apparent in Scotland. Moreover, the tick MLST scheme revealed that Scottish specimens form a clade that is partially separated from other British ticks, with almost no contribution of continental sequence types in this north-westerly border of the tick's natural range. The low diversity of *Ca*. M. mitochondrii, in contrast with previously reported high rates of polymorphism in *I. ricinus* mitogenomes, suggests that the symbiont may have swept across Europe recently via a horizontal, rather than vertical, transmission route.

Wolbachia modifies thermal preference in *Drosophila melanogaster*

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Environmental variation can have profound and direct effects on fitness, fecundity, and host-symbiont interactions. Host-symbiont conflicts may arise from disparities between physiological requirements of *Wolbachia* and those of their hosts. Replication rates of microbes within arthropod hosts, for example, are correlated with incubation temperature but less is known about the influence of host-symbiont dynamics on environmental preference. Hence, we built a custom thermal gradient apparatus and conducted laboratory – based thermal preference (T_p) assays and tested if infection status and genetic variation in endosymbiont bacterium *Wolbachia* affected temperature choice of *Drosophila melanogaster*. We demonstrate that isogenic flies infected with *Wolbachia* preferred lower temperatures compared to uninfected *Drosophila*. Moreover, T_p varied with respect to three investigated *Wolbachia* variants (*w*Mel, *w*MelCS and *w*MelPop). While uninfected individuals preferred 24.4°C, we found significant shifts of -1.2°C in *w*Mel- and -4°C in flies infected either with *w*MelCS or *w*MelPop. We, therefore, postulate that *Wolbachia*-associated T_p variation within a host species might represent a behavioral accommodation to host-symbiont interactions and trigger behavioral self-medication and bacterial titer regulation by the host. This research elucidates fundamental ecological conflicts between *D. melanogaster* and the endosymbiont under laboratory conditions, which may arise in other native, but possibly also *de novo* host-*Wolbachia* associations.

Wolbachia skews social and life history traits and reproductive investment in pharaoh ants

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Wolbachia, a maternally inherited group of bacteria, are widely documented to affect the reproductive behavior, ecology and evolution of their insect hosts. Use of model organisms like *Drosophila* and *Nasonia* have provided insights into mechanisms of inheritance, action and effects of *Wolbachia* infection on the host's fitness. However, due to the solitary nature of these organisms there are limited insights into the effects of *Wolbachia* on multiple social interactions. Almost 30% of ant species are known to be infected with *Wolbachia.* However, there is no understanding of the phenotypic effects of *Wolbachia* in a social organism.

Eusocial model systems like, pharaoh ants or *Monomorium pharaonis*, are an ideal model system to understand the behavioral, genetic, genomic and evolutionary basis of eusociality. They can be readily bred in lab in a controlled manner and show natural differences in *Wolbachia* infection status. *Wolbachia* infection, as a function of queen age, affect total eggs laid by the ant queens, the ant colonies' net productivity and the colonies' reproductive investment. We hypothesize that *Wolbachia* are regulating the life history strategies in pharaoh ants, as a function of queen age. Colonies with young infected queens, in comparison to those with young uninfected queens, invest more in reproduction and have higher net productivity. These colonies produce more number of eggs and produce more number of queens and males for reproduction. However, the reproductive output of these colonies decline faster as the queen ages. Thus infected colonies seem to follow a `live fast, decline young' strategy, whereas uninfected colonies seem to follow a `slow and steady' life history strategy.

Using a series of behavioral experiments, we have characterized the effects of *Wolbachia* on social traits, life history traits and reproductive investment in a social organism to provide insights that have been missing in the field.

Wolbachia, leafhoppers and the dark side of symbiosis

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Obligate endosymbiosis is operationally defined when loss or removal of the endosymbiont from the host results in the death of both. Whereas these relationships are typically viewed as mutualistic, molecular and cellular analysis reveals numerous instances in which these symbiotic relationships are established by alternative, non-mutualistic strategies. The endosymbiont usurps or integrates into core host processes, creating a need where none previously existed. Observations of *Wolbachia*-host interactions at the cellular level suggest many are the result of this addictive form of symbiosis. Here I will discuss these examples and suggest addictive symbiotic relationships and are a likely outcome of all complex evolving systems.

Genomic insights into the pathogenicity of *Rickettsiella* spp., intracellular bacteria of arthropods.

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Gammaproteobacteria of the genus *Rickettsiella*, closely related to *Legionella* and *Coxiella*, are well known as pathogenic bacteria in many arthropods (Bouchon et al. 2011). In woodlice, while *Rickettsiella* was primarily only described as a virulent agent (Bouchon et al., 2011; Cordaux et al., 2007), we showed that some strains may be non-pathogenic (Dittmer et al., 2016; Bouchon et al. 2016). Non-pathogenic strains were also reported in aphids where they act as mutualists conferring benefits to their hosts by protecting against predators (Tsuchida et al. 2010) or a fungal pathogen (Lukasik et al. 2012). *Rickettsiella* was recently found highly abundant in ticks with no evidence of virulence (Duron et al. 2016). However, a strain initially identified as *Diplorickettsia massiliensis* (Mediannikow et al. 2010) but nested in the *Rickettsiella* genus (Leclerque & Kleespies 2012), was later recognized as a human pathogen (Subramanian et al. 2012). *Rickettsiella* therefore constitutes a particularly interesting group to study the evolutionary emergence of pathogenicity. Unfortunately, there are only a very few genomic data for *Rickettsiella*: only one whole and annotated genome of *R. isopodorum* from the woodlouse *Trachelipus rathkei* has been recently published (Wang & Chandler 2016), whereas two draft genomes were available: *R. grylli* isolated from an unidentified woodlouse (GenBank AAQJ00000000) and *D. massiliensis* from the tick *Ixodes ricinus* (Mathew et al. 2012). Interestingly several genomic islands have been identified in *R. isopodorum* but absent in *R. grylli*. By NGS metagenomics approaches, we completed these data with five new genomes of *Rickettsiella* from distinct species of woodlice. Phylogenomics showed that these genomes were closely related to *R. grylli* and *R. isopodorum* but distantly related from *D. massiliensis*, all belonging to a well-defined *Rickettsiella* genus. Comparative genomics allowed us to identify T4SS secretion systems and a high number of putative virulence factors including eukaryote-like domain-containing proteins

Genomics of a *Cardinium-Wolbachia* dual endosymbiosis in a plant-parasitic nematode

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Wolbachia and *Cardinium* are among the most important and widespread of all endosymbionts, occurring in nematodes and most insect and arachnid species, sometimes as coinfections. These symbionts are of significant interest as potential biocontrol agents due to their abilities to affect host biology and reproduction through cytoplasmic incompatibility, sex ratio distortion, or obligate mutualism. The ecological and metabolic effects of coinfections are not well understood. This study examined a *Wolbachia-Cardinium* coinfection in the plant-parasitic nematode, *Pratylenchus penetrans*, producing the first detailed study of such a coinfection using fluorescence in situ hybridization (FISH), PCR, and comparative genomics. Results from FISH and single-nematode PCR showed 123/127 individuals in a focal population carried *Cardinium* (denoted strain cPpe), and 48% were coinfected with *Wolbachia* strain wPpe. Both endosymbionts showed dispersed tissue distribution with highest densities in the anterior intestinal walls and gonads. Phylogenomic analyses confirmed an early place of cPpe compared to *Cardinium* strains in insects and long distance from a sister strain in another plant-parasitic nematode, *Heterodera glycines*. This result, together with a phylogeny of wPpe, support a long history of both *Cardinium* and *Wolbachia* in plant-parasitic nematodes, perhaps involving longstanding coinfection. Genome features showed cPpe was missing biotin and lipoate synthetic capacity and a plasmid present in other strains, despite having a slightly larger genome (1,358,214 bp) and more predicted proteins (1,131) compared to other sequenced *Cardinium* strains. The larger genome revealed expansions of gene families likely involved in host-cellular interactions, and more than 2% of the genes of cPpe and wPpe were identified as candidate horizontally transferred genes, with some of these from eukaryotes, including nematodes. Based on the genomes of these two coinfecting symbionts, a model of the *Wolbachia-Cardinium* interaction is proposed with possible complementation in function for pathways such as methionine and fatty acid biosynthesis and biotin transport.

Using metabolic networks to characterize the symbiosis between *Wolbachia* and filarial nematodes, and identify novel drug targets

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Filarial nematodes represent one of the leading causes of disability in the developing world. Many filarial worm species, including *Brugia malayi*, one of the causative agents of lymphatic filariasis, have an obligate endosymbiotic relationship with the alpha-proteobacteria *Wolbachia*. To better understand the molecular interplay between these two organisms, we profiled the transcriptomes of *B. malayi* and *Wolbachia* across the life cycle of the parasite using dual RNA-seq. This allowed us to pinpoint functional pathways that are putatively involved in this essential symbiotic relationship and provided by the developmentally regulated co-expression of nematode and bacterial genes.

We are currently focusing on molting of the worm from L3 to L4, the molt which marks the establishment of infection in the human host and is characterized by a large expansion of the *Wolbachia* population. We have profiled the co-expressed pathways associated with molting as well as how those pathways are affected by either blocking molting of the worm or blocking bacterial expansion.

In parallel we used these transcriptomic data in combination with genomic data to characterize the nematode and endosymbiotic relationship at the metabolic level using Flux Balance Analysis to identify choke points that could be exploited for therapy. To better inform our metabolic model, we have also profiled the metabolome of both *Brugia malayi* and *Wolbachia* over the life cycle of the worm. By creating a draft metabolic network for *B. malayi* and *Wolbachia*, and by using *in silico* knockouts, we have identified pathways that are possibly necessary for development and fitness, and how these pathways are influenced by the presence or absence of *Wolbachia*. These predictions are now being tested using RNAi and small molecule inhibitors in order to validate those that can be further developed as novel drug targets.

The Stage-Specific Transcriptome of the *Wolbachia* Endosymbiont of *Brugia malayi w*Bm through the Nematode Lifecycle

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Brugia malayi is one of three filarial nematodes that cause human lymphatic filariasis. We used RNASeq, EdgeR, WGCNA and numerous laboratory methodological improvements to identify the transcriptomic alterations in *B. malayi,* its *Wolbachia* endosymbiont *w*Bm, and its invertebrate host, *Aedes aegypti,* across 15 time points during the *Brugia* life cycle. While we identified more than a dozen expression profiles for *B. malayi* across the life cycle, there were far fewer in *w*Bm. Of the 839 protein-coding genes identified in *w*Bm, only 336 were differentially expressed. When WGCNA is used to cluster the differentially expressed genes into modules based on similarities in expression profile, only two modules were identified with >50 genes. The largest module consists of 161 genes and is differentially expressed in the three vector samples taken 18 hpi, 4 dpi, and 8 dpi of *B. malayi* relative to the infected gerbil samples. This module is significantly enriched for structural constituents of the ribosome, suggesting that upon the transition of *B. malayi* to the vector host, *w*Bm increases its rate of translation. The only other module with significantly enriched functional terms consists of 33 genes enriched in protein folding that are upregulated specifically in adult male *B. malayi*. Additionally, we find that the expression of the 6S RNA, a noncoding RNA whose expression correlates with bacterial replication rates, increases as *B. malayi* matures from L3 to the adult and embryo life stages. Upon the embryo maturing to microfilaria, the expression of the 6S RNA drops drastically. Based off this analysis, we propose that rather than altering the expression of specific metabolic pathways, the differential expression observed in *w*Bm across the *B. malayi* life cycle is primarily based on altering its rates of replication, transcription, and translation, which may not be surprising for obligate intracellular bacteria with very limited environmental variation.

The effect of *Wolbachia* on gene expression in *Drosophila paulistorum*

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The Neotropical superspecies *Drosophila paulistorum* (Diptera: Drosophilidae) consists of at six reproductively isolated semispecies, each harboring a closely related mutualistic *Wolbachia* strain. Although wildtype flies are isolated from other semispecies by both pre- and postmating incompatibilities, recent studies have shown that mating and successful offspring development can be achieved after flies are treated with antibiotics for reduction of the *Wolbachia* infection titer. This suggests the bacteria not only prevent embryonic development through cytoplasmic incompatibility, but also affect mate choice, possibly through interference in pheromone production and recognition. The present study investigates *Wolbachia*'s influence on differential gene expression of *D. paulistorum* using RNA-seq data from heads and abdomens of both sexes of three semispecies, namely Amazonian (AM), Centro-American (CA) and Orinocan (OR). Both naturally infected wild type flies and specimens submitted to antibiotics treatment and subsequent gut flora restoration were sampled. Our data allowed good quality transcriptome reconstructions for each semispecies, which we used for performing a differential expression analysis. The OR semispecies, which hosts high titer *Wolbachia* infection, generated the largest number of differentially expressed transcripts, 1872, followed by AM (300) and CA (210), both of which have very low titer infections. A GO-term enrichment analysis allowed us to identify biological processes overrepresented in global, tissue-specific, sex-specific and condition-specific patterns. Some of the main functions seen on a global scale are the down-regulation of antimicrobial peptides, increased expression of metabolic genes, and, with a few exceptions, up-regulation of genes related to pheromone production and reception. Other interesting observations include up-regulation of reproduction-related genes in female abdomens, down regulation of muscle-associated genes in abdomens and up-regulation of translation-related genes in female heads. Our results show that *Wolbachia* affects genes potentially related to pre- and post-mating isolation in *D. paulistorum*, corroborating a possible influence in host speciation.

Gaining a mechanistic insight into *Wolbachia*-mediated virus resistance

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In arthropods, presence of *Wolbachia* is associated with resistance against RNA viruses. In addition, *Wolbachia* is able to cause reproductive manipulations in its host, which it uses to spread across insect populations, making it a promising tool to prevent dissemination of arthropod-transmitted diseases. Despite successful trials using *Wolbachia*-transfected mosquitoes for the control of Dengue, Zika, and Chikungunya, we do not know the molecular mechanisms of pathogen blocking. We developed a model system using the fruit fly *Drosophila melanogaster* and the *w*Mel *Wolbachia* strain to show that the *Drosophila* RNA methyltransferase (Dnmt2) is required for resistance against multiple positive-sense single-stranded RNA viruses. We found that *Wolbachia*-infected cells constitutively upregulate Dnmt2, creating an environment refractory to viral replication, in turn producing virions that are less infectious in vertebrate cells. Reduction in virus infectivity was determined to be due to impaired viral genomic RNA produced in the presence of *Wolbachia*. Given that Dnmt2 is an RNA methyltransferase, we explored methylation patterns of viral genomic RNAs to see if this could explain the antiviral effect. Viral genomes derived from *Wolbachia*-infected cells were found to be significantly more methylated than those derived from *Wolbachia*-free cells. However, it remains to be determined which particular methyl-cytosine sites on the viral genome are critical for achieving this antiviral effect. Determining how *Wolbachia* causes inhibition of RNA viruses will expand our knowledge of its influence on host biology while contributing to the development of *Wolbachia*-based vector control initiatives.

Insect symbionts as bio-control agents against plant pathogens

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Phloem restricted bacterial pathogens pose a major threat in many agricultural crops. Because the conventional application of chemical sprays is inefficient, endophytes have been suggested as a potential reservoir for innovative control approaches. We hypothesized that insects that are involved in the transmission of plant pathogens may harbor microbes that affect disease agents. The study reported is focused on a bacterium that was isolated from the planthopper *Hyalesthes obsoletus* (Hemiptera: Cixiidae), the insect vector of Bois noir disease. The isolate belongs to a novel bacterium from the family Xanthomonadaceae, for which the name *Frateuria defendens* sp. nov. is proposed. This isolate could be introduced into a number of healthy and infected crop plants, and its presence in plant tissues was confirmed up to four weeks post inoculation. In the presence of the isolate symptoms of disease-causing bacteria such as *Phytoplasma* and *Liberibacter* were markedly reduced in both laboratory and field experiments. Taken together, the results demonstrate that insects that serve as vectors for plant pathogens may be a useful source for potentially beneficial bacteria.

Artificial selection on *Wolbachia*-mediated blocking of dengue virus

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We have recently demonstrated family level variation in the trait of *Wolbachia* mediated blocking of dengue virus in *w*Mel infected *Aedes aegypti*. Here we performed artificial selection to both increase and decrease the strength of blocking as measured by total viral load in the insect. We were able to successfully shift the phenotype in both directions and have examined in parallel concomitant effects on host fitness and *Wolbachia* levels. We also carried out whole genome sequencing in the selected lines to identify any associated changes in the *Wolbachia* and the mosquito. These data reveal the capacity for the blocking trait to evolve and shed light on its possible mechanism.

Developing *Wolbachia* to combat mosquito-borne diseases

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Diseases transmitted by mosquitoes include dengue, Zika and malaria. Due to insufficiency of the traditional approaches, such as drugs, vaccines and chemical insecticides, in controlling these diseases, great efforts have been put to develop novel solutions to save human life. The ability to establish novel *Wolbachia* symbiosis in mosquito vectors enable us to utilize *Wolbachia*-mediated cytoplasmic incompatibility and pathogen interference for reducing infectious mosquito biting. I will introduce our recent work on understanding the molecular mechanisms of *Wolbachia*-mediated pathogen (virus and *Plasmodium*) interference and the roles of mosquito innate immunity in establishing novel symbiosis in mosquitoes. To accelerate translation of *Wolbachia* into a tool for disease control, we have established a mosquito factory and developed a field trial through releasing five million of *Wolbachia*-infected male mosquitoes per week in Guangzhou China. This resulted in nearly eliminating dengue mosquito population with suppression consistently maintained in the field. Further field trials using different release strategies are also being developed to combat dengue, Zika or Malaria through collaborations with governments of endemic countries and industrial partners.

Flies and *Wolbachia* talk using *Wnt* sign(aling) language

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Endosymbiotic bacteria and their host cells engage in a complex communication process that results in a long-term association and preferential infection of specific cell types. This preferential infection or certain tissues or cells is known as tropism. The signals that promotes *Wolbachia* tropism is not well understood. Here we show that *Drosophila* canonical Wnt signaling, a key regulator of developmental patterning and cell proliferation is a key host signal for *Wolbachia* tropism. *Wnt* signaling modulates *Wolbachia* levels in specific cell types, including stem cell niches in the testis, ovary and ovarian polar cells. Knockdown of Wnt signaling caused a decrease in *Wolbachia* density whereas an upregulation of Wnt signaling led to an increase in *Wolbachia*. Our work provides a novel mechanism for *Wolbachia* intracellular accumulation.

Wolbachia and host germline components compete for kinesin-mediated transport to the posterior-pole of the *Drosophila* oocyte

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Widespread success of the intracellular bacterium *Wolbachia* across insects and nematodes is due to efficient vertical transmission and reproductive manipulations. Many strains, including wMel from the fruit fly *Drosophila melanogaster*, exhibit a specific concentration to the germ-plasm at the posterior pole of the mature oocyte, thereby ensuring high fidelity of parent-offspring transmission. Transport of *Wolbachia* to the pole relies on microtubules and the plus-end directed motor kinesin heavy chain (KHC). However, the mechanisms and protein(s) mediating *Wolbachia's* association with KHC remain unknown. Here we show that reduced levels of the host scaffold/linker protein APLIP1 reduces *Wolbachia* transport and concentration at the posterior pole of the oocyte. These results suggest that APLIP1 is involved in securing *Wolbachia* to KHC. Surprisingly, reducing levels of the canonical linker protein KLC dramatically increased levels of *Wolbachia* at the oocyte's posterior. This indicates that KLC and associated host cargo outcompete *Wolbachia* for association with a limited amount of KHC motor proteins. Furthermore over-expression of KHC causes similarly increased levels of posteriorly localized *Wolbachia*, but has no effect on levels of Vasa, a germ-plasm component that also requires KHC for posterior localization. Thus, *Wolbachia* transport is KHC-limited because these bacteria are likely outcompeted for binding to KHC by host cargo/linker complexes. These results reveal a novel host-symbiont interaction that underscores the precise regulation required for an intracellular bacterium to co-opt, but not disrupt, vital host processes.

A taxonomic analysis of the Rickettsiales using core genome alignments to recommend species guidelines

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Despite a plethora of genomes and genomic tools for high resolution taxonomic classifications, the classification systems for most of the bacteria in the order Rickettsiales still rely on MLST-based approaches, which at best account for <1% of the nucleotides present in any given bacterial genome. In this study, we constructed core genome nucleotide alignments (CGAs) to examine nucleotide identity matrices and infer phylogenomic relationships for each Rickettsiales genus, including the *Rickettsia*, *Orientia, Ehrlichia, Neoehrlichia, Anaplasma, Neorickettsia,* and *Wolbachia.* We find that applying a 96.8% nucleotide identity threshold to CGA-based nucleotide identity matrices can be used to delineate species while genera can be deduced by assessing whether the input genomes can form a core genome alignment. Further testing reveals that these criteria can largely reproduce genus and species boundaries for many other bacterial genera including the *Arcobacter, Caulobacter, Erwinia, Neisseria, Polaribacter, Ralstonia,* and *Thermus*, suggesting that this technique and criterion can be widely applied to diverse bacterial taxa. Within the Rickettsiales, the application of these guidelines indicates that *Anaplasma phagocytophilum* belongs to a separate genus from the other *Anaplasma* while *Neorickettsia helminthoeca* belongs to a separate genus from the other *Neorickettsia*. Furthermore, these guidelines support collapsing the two dozen different *Rickettsia* species to only nine species, which indicates that there is currently an overabundance of unique species designations in the *Rickettsia*. For *Wolbachia* endosymbionts, supergroups A and B each appear to be their own species, whereas each of the filarial nematode supergroups would contain multiple species. We hope that these results and the work of others on this topic will spearhead a community wide effort at this meeting to assign species designations to *Wolbachia* species with at least one high quality, preferably complete, whole genome sequence.

Functional genomics approaches to study the non-protective phenotype of the endosymbiont, *Sodalis glossinidius.*

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African trypanosomes cause trypanosomiasis in both animals ("nagana") and humans ("sleeping sickness"). Nagana is associated with an estimated annual $4.5 billion loss to livestock production, in Sub-Saharan Africa. The World Health Organization (WHO) estimates that 60 million people in Africa are at risk of contracting sleeping sickness (about 40% of the continent's population). In order to establish effective control, it is essential that we understand the transmission cycle of the parasite in the insect vector; trypanosome infection rates of tsetse in the wild are normally low (<1%), as many parasites entering the midgut die. Natural refractoriness of tsetse to trypanosomes is variable and has a number of contributing factors (e.g. tsetse age, sex and genetic background). One of the most important determinants of tsetse fly susceptibility to trypanosome infection is the presence of the midgut endosymbiont, *Sodalis glossinidius*; whilst bacterial symbionts and gut microbiota have in other systems been shown to reduce vector competency, *S. glossinidius* has the opposite effect and enhances the infection rate. The central hypothesis of my Ph.D. is that the symbiotic interactions, theoretically as a result of the pseudogenes and virulence-associated genes, within the tsetse host results in the fly being more susceptible to trypanosome infection. RNA sequence and Tn5 mutagenesis has been used in order to identify *S. glossinidius* candidate genes/interactions involved in symbiosis and by extension, the role of *S. glossinidius* in increasing trypanosome transmission.

Recurrent replacement of eroding ancient endosymbiont by domesticated fungal pathogens

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Diverse insects are associated with ancient bacterial symbionts, whose genomes have often suffered drastic reduction and degeneration. In extreme cases, such symbiont genomes seem almost unable to sustain the basic cellular functioning, which comprise an enigmatic issue of evolutionary biology. Here we report an insect group wherein ancient symbiont lineage suffering massive genome erosion has experienced recurrent extinction and replacement by host-associated pathogenic microbes. Cicadas are associated with the ancient bacterial co-obligate symbionts *Sulcia* and *Hodgkinia*, whose streamlined genomes are specialized for synthesizing essential amino acids and some vitamins, thereby enabling host's living on nutritionally poor plant xylem sap. However, our inspection of 24 Japanese cicada species revealed that, while all species possessed *Sulcia*, only 9 species retained *Hodgkinia*, and their genomes exhibited substantial structural instability. The remaining 15 species lacked *Hodgkinia* and instead harbored yeast-like fungal symbionts. Detailed phylogenetic analyses uncovered repeated *Hodgkinia*-fungus and fungus-fungus replacements in cicadas. The fungal symbionts were phylogenetically intermingled with cicada-parasitizing *Ophiocordyceps* fungi, identifying entomopathogenic origins of the fungal symbionts. Most fungal symbionts of cicadas were uncultivable, but the fungal symbiont of *Meimuna opalifera* was exceptionally cultivable, possibly because it is at an early stage of fungal symbiont replacement. Genome sequencing of the fungal symbiont revealed its metabolic versatility presumably capable of synthesizing almost all amino acids, vitamins and other metabolites, which is more than sufficient to compensate for the *Hodgkinia* loss. These findings highlight a straightforward ecological and evolutionary connection between parasitism and symbiosis, which may provide an evolutionary trajectory to renovate deteriorated ancient symbiosis via pathogen domestication. The pathogen-derived symbiont recruitment may represent a general mechanism underpinning parasitism-symbiosis evolutionary continuum, compensation of symbiont genome erosion, and diversification of host-symbiont associations.

Colonization of *Wolbachia* Endosymbionts within *Drosophila* Ovarioles

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Vertical transmission requires successful *Wolbachia* transmission to developing eggs. This is achieved through colonization of developing germline cells, by initial loading from the germline stem cell, horizontal invasion and bacterial replication. Little is known about the respective contributions of each input to the process of germline colonization, however. The timetable of developmental progression, from germline stem cell through stage 10 egg chambers, is known. Thus, comparing *Wolbachia* titers between stem cells, stage 4 egg chambers, and stage 10 egg chambers is expected to illuminate the timing of *Wolbachia* titer increases, providing data to test alternative mechanistic hypotheses. We quantified *w*Mel titer at designated stages of *Drosophila melanogaster* egg chamber development using confocal microscopy. Individual ovarioles containing cells at each of the three targeted developmental stages were analyzed and *Wolbachia* counts at each stage were validated by multiple statistical approaches. The results indicated that a simple exponential growth model is not sufficient to account for *Wolbachia* titer increases across oogenesis. To further address whether *Wolbachia* titer increases in oogenesis are due to horizontal invasion, bacterial replication, or both, the morphology and distribution of *Wolbachia* within the egg chambers are being assessed. Overall, we expect these results to elucidate the germline colonization by *Wolbachia* as an integrated, stepwise process

Identification of the genetic bases of new overproliferative *Wolbachia* variants

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Bacteria of the genus *Wolbachia* are maternally transmitted endosymbionts of several arthropod species. They are often parasitic and able to induce several reproductive manipulations in their hosts, contributing to their own fitness. They also have the ability to block viral replication in *Drosophila* and in mosquito vectors of human arboviruses. Because of this, *Wolbachia*-carrying mosquitoes are currently on trial as a self-sustaining approach to block transmission of viruses. Despite this, the molecular bases of *Wolbachia*-host interaction are yet not fully understood. This is mainly caused by the lack of genetic manipulation systems for this bacterium. To overcome this limitation we employed a new screening strategy to identify new *Wolbachia* variants that overproliferate. Our approach was successful and allowed us to isolate two such mutants. We found that Octomom was amplified in one of the mutants, confirming previous results demonstrating that Octomom copy number determines wMelPop titers. We also identified the mutation conferring the overgrowth phenotype in the second line and we have characterized this variant phenotypically. Taken together, our results demonstrate the feasibility of a forward genetic screen in *Wolbachia* and identified a new *Wolbachia* mutation leading to overproliferation. This new approach can be extended to further understand *Wolbachia* biology and adapted to study other endosymbiont-host systems.

Novel *Wolbachia-Drosophila* interaction involves host translation

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Wolbachia infect a remarkable range of insect hosts, but the interactions between *Wolbachia* and their hosts are not fully characterized. Two outstanding questions include: which host cellular compartments serve as *Wolbachia* niches, and which host systems affect *Wolbachia* load dynamics. To address these questions, we used the *Wolbachia*/*Drosophila* model that provides a genetically tractable system for studying host-pathogen interactions. First, using serial focused ion beam electron microscopy we three-dimensionally reconstructed *Wolbachia* intracellular niches and associated host membrane compartments within *Drosophila* gonads as well as developing embryos. We found that *Wolbachia* reside intracellularly within modified host endoplasmic reticulum (ER) niches. Second, we used a *Wolbachia*-infected *Drosophila* cell line JW18 to perform an unbiased *Drosophila* whole genome RNAi screen taking advantage of a novel high-throughput fluorescence in situ hybridization (FISH) assay to detect changes in *Wolbachia* levels. 1117 genes altered *Wolbachia* levels when silenced by RNAi of which 329 genes increased and 788 genes decreased the level of *Wolbachia.* Validation of hits included in depth secondary screening using *in vitro* and *in vivo* RNAi, *Drosophila* mutants, and qPCR. A diverse set of host gene networks were identified to regulate *Wolbachia* levels including protein anabolism and catabolism, translation and cell cycle. We found an unexpected role of host translation components such as the ribosome and translation initiation factors in suppressing *Wolbachia* levels both *in vitro* using RNAi and *in vivo* using RNAi, mutants, and chemical-based translation inhibition assays. This work provides the first evidence for *Wolbachia*-host translation interaction. Furthermore, our three-dimensional *Wolbachia* niche reconstruction analyses confirmed the interaction between *Wolbachia* and the host ER compartment and strengthens our general understanding of the *Wolbachia*-host intracellular relationship.

Regulation of *Wolbachia* by host autophagy across multiple cell-types in *Drosophila melanogaster*

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Autophagy is a conserved intracellular degradation pathway involved in recycling cytoplasmic constituents, including protein aggregates or damaged organelles. Beyond its role in cytoplasmic maintenance, autophagy has been shown to act as an innate immune response targeting intracellular pathogens to the lysosome for degradation. While much work currently investigates autophagic-pathogen interactions, less is known about how autophagy interacts with endosymbionts including *Wolbachia*. Previous studies showed that flies treated with rapamycin, a known autophagy inducer, led to decreased *Wolbachia* density in larva but increased density in the germline. This indicated that *Wolbachia* densities may be modulated by host autophagy in a cell-type dependent manner. Expressing RNAi against multiple autophagy proteins in a cell-type specific manner we show increased *Wolbachia* density in the hub and polar cells, a somatic cell population in the testis and female germline respectively. Further investigation into how autophagy negatively regulates *Wolbachia* in the hub reveals selective autophagy proteins are implicated in the degradation of *Wolbachia* in these cell types. Conversly, preliminary work identifies of a subset of autophagy proteins essential for non-selective autophagy that positively effect *Wolbachia* densities, revealing how multiple autophagy pathways can interact with *Wolbachia* differently in the same cell. Lastly, knockdown of autophagy in the germline reduced germline *Wolbachia* density indicating how *Wolbachia* are able to utilize autophagy in the germline to reside at higher densities. Overall, we characterized how *Wolbachia*, an obligate endosymbiont interacts with host autophagy differently across multiple cell types and further describe how different types of autophagy within the same cell-type regulate *Wolbachia* density differently. This research provides novel insights into host-endosymbiont interactions including host degradation of endosymbionts by autophagy and how *Wolbachia* uses alternative forms of this process for their benefit.

Optimizing *Wolbachia* infection and maintenance in mosquito cell lines

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Development of cell lines that readily establish and maintain productive *Wolbachia* infections will facilitate genetic manipulation of *Wolbachia* and enhance its utility for biological control of pest species. Factors that favor establishment of long-term persistent *in vitro* infections are poorly understood. Although infection can be established in mitomycin-C treated, non-dividing host cells, maintenance of a persistent infection with high yields of infectious *Wolbachia* requires the host cell to balance its own requirements for sustained growth and division with metabolic demands of the intracellular bacterium. Although some strains of *Wolbachia* readily grow in cultured cells for a short time, and a few strains such as *w*Str and *w*AlbB can be persistently maintained, strains such as *w*Pip have yet to be propagated in cell lines. In *Aedes albopictus* cells persistently infected with *w*Str, proteomic analyses indicate that *Wolbachia* proteins involved in ribosome structure, biogenesis and translation are highly co-expressed with a *Wolbachia* footprint dominated by chaperones, stress response and cell membrane proteins. Low expression of *Wolbachia* proteins involved in amino acid and carbohydrate metabolism is consistent with its requirements for host metabolic precursors. The host cell shows upregulated production of amino acids, lipids and cofactors that complement *Wolbachia*'s metabolic deficiencies, and downregulation of transcription, ribosome biosynthesis, DNA repair and cell division. Flow cytometry provides a relatively simple method for monitoring abundance of *Wolbachia* and host cells in samples treated under various culture conditions. Conditions that optimize infection of naïve host cells and enhance production of infectious *Wolbachia* will be described.

Effects of temperature on *Cardinium*-induced cytoplasmic incompatibility in a parasitoid

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Terrestrial arthropods, including insects, commonly harbor heritable intracellular symbionts. These symbionts cannot survive outside the host and often confer novel beneficial phenotypes to the host to maintain stable vertical transmission. While many heritable symbionts are mutualistic, symbionts can also maintain vertical transmission through direct manipulation of host reproduction to favor infected female progeny via mechanisms like cytoplasmic incompatibility (CI). The efficacy of symbiont-induced effects often varies with changing environmental conditions, potentially leading to destabilization of the symbiosis. For example, increased temperatures can reduce the titer of a common reproductive manipulator, *Wolbachia*, and the strength of its conferred CI. Another manipulative symbiont, *Cardinium*, infects ~ 6% of Arthropods, including the whitefly parasitoid, *Encarsia suzannae* (Hymenoptera: Aphelinidae); this symbiont also induces CI in its host, however there appears to be little homology between the CI mechanisms of *Cardinium* and *Wolbachia*. Currently, little is known concerning the impact of temperature on the *Cardinium-E. suzannae* association, including whether temperature disrupts the CI phenotype. Here, we investigated the effect of warm and cool temperatures on bacterial titer and CI strength in the *Cardinium-E. suzannae* system.

Endosymbionts modulate sex ratio bias in *Pezothrips kellyanus* in a temperature-dependent way

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Sex ratios can be genetically determined or environmentally manipulated. In many arthropod species sex ratios are also distorted by selfish genetic elements, including maternally inherited endosymbionts. Their manipulations can increase reproductive fitness of infected female individuals and thus infection rates in host populations. Individuals of Australian populations of *Pezothrips kellyanus* (Thysanoptera) are co-infected with *Cardinium* and *Wolbachia*. Both manipulate the thrips' arrhenotokous reproduction through the induction of cytoplasmic incompatibility. Previously, the sex ratio of this species was found to be temperature-dependent, with a female-biased sex ratio at low temperature, an even sex ratio at moderate temperatures and a male-biased sex ratio at high temperature.

In our study we aimed to uncover the role of the endosymbionts in this temperature-dependent sex ratio pattern. We contrasted offspring sex ratios of infected and uninfected mated pairs at 20°C and 25°C and detected sex ratio shifts that were due to *Cardinium* and *Wolbachia* co-infections. The infected population produced female-biased offspring at both temperatures, with a stronger female bias at 20°C. In contrast, the uninfected population produced offspring with a fairly even sex ratio across both temperatures. There was a bimodal pattern underlying the sex ratio bias, with some females only producing male offspring when mated with compatible males. Intriguingly, the proportion of mated females producing male-only offspring was higher for uninfected pairs. Some infected mated females also produced male-only broods at 25°C but this was not seen at 20°C. Generally it can be expected that haplodiploid females reproducing by arrhenotoky produce female-biased broods when mated with compatible males. However, it appears that *P. kellyanus* suffers from fertilisation issues that are alleviated by the presence of endosymbionts in a temperature-dependent way. This may contribute to the reproductive success of the host species and the maintenance of endosymbiont infections in host populations.

The maternal effect gene *Wds* controls *Wolbachia* titer in *Nasonia*

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Maternal transmission of intracellular microbes is pivotal in establishing long-term, intimate symbioses. For germline microbes that exert negative reproductive effects on their hosts, selection can theoretically favor the spread of host genes that counteract the microbe's harmful effects. Here, we leverage a major difference in *Wolbachia pipientis* titers between closely-related wasp species with forward genetic, transcriptomic, and cytological approaches to map two quantitative trait loci that suppress bacterial titers via a maternal effect. Fine mapping and knockdown experiments identify the gene *Wolbachia density suppressor* (*Wds*), which dominantly suppresses bacterial transmission in *Nasonia* from mother to embryo. *Wds* evolved by lineage-specific non-synonymous changes driven by positive selection. Collectively, our findings demonstrate that a genetically simple change arose by Darwinian selection in less than a million years to regulate maternally transmitted bacteria via a dominant, maternal effect gene.

Genetic determinism of the high diversity of Cytoplasmic Incompatibility induced by *Wolbachia* in the mosquito *Culex pipiens*

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All *Culex pipiens* mosquitoes are infected with *Wolbachia* (*w*Pip), responsible for an unrivalled diversity of CI phenotypes. Functional transgenic studies have recently pointed out the *cidA-cidB* operon of *Wolbachia* as responsible for CI between infected *Drosophila* males and uninfected females.

We investigated the genetic basis of CI diversity by studying the polymorphism of *cidA-cidB* genes between different *w*Pip strains inducing different CI phenotypes in *Culex pipiens*. We showed that the remarkable CI diversity in *C. pipiens* is linked to the presence of several copies of the *cidA-cidB* operon within each *w*Pip genome, which undergoes diversification through recombination events. The screening of *cidA-cidB* genes repertoires of more than 200 isofemale lines obtained from *C. pipiens* larvae collected worldwide revealed specific repertoires variations that matched CI phenotypes. Studies in different geographical locations confirmed the implication of CidB as a modification (Mod) factor while CidA does not seem implicated in this function. The polymorphism between the different *cidA-cidB* copies occur in putative domains of reciprocal interactions. Such observation is consistent with the hypothesis of a toxin–antitoxin system in which both genes co-diversify.

Too many cooks spoil the brew? Co-infection context influences reproductive manipulation of a linyphiid spider

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Given the prevalence of endosymbionts that manipulate host reproduction among arthropods, it is inevitable that these symbionts must occasionally interact with one another over evolutionary time, either within a shared host, or through differentially infected hosts within a population. Yet contemporary examples of such interaction appear to be relatively rare among insects. Here, I will make the case that symbiont-symbiont interactions may be more common among non-insect arthropods, such as spiders, and that these systems are useful for understanding interactions among symbionts that each employ different reproductive manipulations of a shared host. We have surveyed the symbiont community of multiple spider species, and found that approximately half the species examined show evidence of either multiple infection within the same individual, or mixed infection by different symbionts within the same population. Within one spider species, *Mermessus fradeorum* (Linyphiidae), we experimentally documented that different matrilines experienced either bacterially-mediated feminization or cytoplasmic incompatibility, and that spiders exhibiting both types of manipulations were present in the same field population. We have since characterized the microbiome of *M. fradeorum*, and found 5 endosymbionts present within the population: a *Rickettsia*, three strains of *Wolbachia*, and a *Rickettsiella*. Spiders co-infected with all five symbionts were feminized, but spiders infected with various subsets of the symbionts were not, which suggests that co-infection context influences expression of the phenotype. Conversely, the feminized spiders were not able to fully rescue cytoplasmic incompatibility, even though they were infected with the causative symbiont. We hypothesize that there is a tradeoff between feminization and ability to rescue CI based on relative symbiont titer, and that instability of phenotype expression permits persistence of incompatible reproductive manipulations within the host population.

The rise and fall of a transformational bacterial symbiont in an invasive insect herbivore

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Maternally-inherited symbionts generally spread in host populations by increasing host fitness, or by biasing host sex towards females. The invasive globally-distributed sweetpotato whitefly species complex, collectively known as *Bemisia tabaci*, has a diverse assemblage of maternally inherited, facultative bacterial symbionts, among which *Rickettsia* is common worldwide. We found that in the Southwestern USA in the species known as “B” or "MEAM1," *Rickettsia* swept from 1% to 97% frequency in a period of just six years, from 2000-2006. In laboratory assays of whiteflies with and without *Rickettsia* in a homogenous genetic background, *Rickettsia*-infected (R+) whiteflies showed greater performance and greater female-biased sex ratios than uninfected whiteflies. In a second genetic line, *Rickettsia* appeared to confer fewer relative benefits to infected whiteflies, and introgression of the two genetic lines indicated that host nuclear genotype influenced the *Rickettsia*-whitefly phenotype. Interestingly, while *Rickettsia* frequencies stayed high in the field for at least 5 years, from 2006-2011, in 2015, the frequency of *Rickettsia* had dropped to about 60% where it has remained for the last two years. Isolines established from the field in 2016 showed similarly high rates of vertical transmission as in earlier studies, but lower *Rickettsia* titer on average, and no longer any relationship between *Rickettsia* infection and either performance or sex ratio. These results show symbiont frequencies in host populations can change dramatically within a few years, and reflect changes in the phenotype of the symbiont-infected host. Host population structure appears to play an important role in these dynamics.

Does getting defensive get you anywhere? - Dissecting the causes of rapid symbiont dynamics in the pea aphid

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Heritable symbionts are common across insects, and many infect their host species at intermediate frequencies. While a range of natural processes may prevent symbiont loss or fixation, balancing selection acting at the host level, provides one intriguing possibility. In the pea aphid, lab-based findings suggest that defensive benefits of an anti-parasitoid, protective bacterial symbiont, *Hamiltonella defensa*, are counter-balanced by costs when parasitoids are rare or absent. If balancing selection takes this form, one would expect natural *H. defensa* dynamics to track seasonally varying parasitoid pressures. Through biweekly sampling across a six month timespan, we find little evidence for such tracking, noting instead that *H. defensa* frequencies are highest when parasitoids are sparse. Rapid and large-magnitude *H. defensa* dynamics were more strongly correlated with other pressures, suggesting uncharacterized benefits of this symbiont. Consistent with these patterns was our discovery of only modest parasitoid effects on *H. defensa* frequencies, under controlled field cage experiments. Furthermore, these symbionts do not show heightened enrichment among pea aphids surviving times of high parasitoid attack. This argues against *H. defensa* presence/absence polymorphism as a major source of defensive variability under the studied conditions. In looking to the future, we see a need to examine parasitoid-symbiont specificity as a driver of natural symbiont dynamics. Symbiont frequency declines during the overwintering period, transmission failure during asexual generations, and potential competition with other symbionts must also be invoked in accounting for natural dynamics. To conclude, the pea aphid system is an optimal model for lab and field studies on symbionts' roles in host adaptation. Their seven heritable symbionts are remarkably dynamic, with capacities for large-magnitude shifts that unfold over timescales of just one-to-two host generations. But lessons learned from years of lab research seem to now require supplementation from experiments informed by real world conditions.

Evolution and specificity in the *Drosophila-Spiroplasma* defensive symbiosis

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Although we now know that many maternally transmitted bacterial endosymbionts protect their hosts against a wide range of natural enemies, the mechanism of protection is still not well understood. *Spiroplasma* bacteria infect a wide range of invertebrates, and include diverse pathogens, gut commensals, and inherited symbionts. Some inherited *Spiroplasma* distort sex ratios via male-killing; host protection is also common. We have been studying associations between *Spiroplasma* strains that protect various *Drosophila* species against infection by parasitic nematodes and parasitic wasps. We found that the genomes of defensive *Spiroplasma* contain a diverse repertoire of toxins called ribosome-inactivating proteins (RIPs), related to Shiga toxins of enterohemorrhagic *E. coli*, and that toxins are frequently gained and lost across *Spiroplasma*. Nematode and wasp ribosomes show characteristic signatures of attack by RIPs, implicating these toxins as major players in defense. We speculate that toxin diversity and evolution play an important role in specificity against different enemies.

Wolbachia effects on arbovirus infections in mosquitoes are dependent on the mosquito species, the *Wolbachia* strain, and the viral pathogen

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The bacterial symbiont Wolbachia is known to reduce pathogen infection and transmission in vector arthropods, leading to trials examining Wolbachia for vector-borne disease control. However, pathogen suppression is not universal; Wolbachia strains have been demonstrated to enhance rather than suppress pathogens in a variety of natural and artificially infected arthropod hosts. Here, we investigated the effects of multiple Wolbachia strains (wAlbB and wMel) on flavivirus (West Nile and Zika viruses), alphavirus (O'nyong nyong, Sindbis, and Mayaro viruses), and bunyavirus (Rift Valley Fever virus) infection in Culex and Aedes mosquitoes. We show that Wolbachia may suppress, enhance, or have no effect on virus infection depending on the mosquito host, the Wolbachia strain, and the pathogen. These data indicate that the suppressive effects of Wolbachia infection on arboviruses are not universal, but rather depend on the characteristics of the specific symbiont-vector-pathogen system in question. These results have important implications for the deployment of Wolbachia-infected mosquitoes into field populations for disease control.

Effect of naturally occurring *Wolbachia* in *Anopheles gambiae* mosquitoes on *Plasmodium falciparum* malaria transmission

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Wolbachia is a genus of gram-negative endosymbiotic proteobacteria that are vertically transmitted and commonly found in a wide range of arthropods; they do not, however, naturally infect *Aedes aegypti* or *Anopheles* mosquitoes. Some strains of *Wolbachia* protect insect hosts from viral infections, and transinfection with *Wolbachia* protect *A. aegypti* against dengue and other flaviviruses. *Wolbachia* also reduces mosquito susceptibility to other non-viral pathogens like *Plasmodium* but attempts to transinfect *A. gambiae* with *Wolbachia* have not been successful. However, *Anopheles gambiae* mosquitoes collected in Burkina Faso naturally infected with a new strain of *Wolbachia* were recently identified. In this work, *An. gambiae* and *An. coluzzii* were collected in Mali, in 2010 and 2015, and screened for the presence of *Wolbachia*. A new naturally-occurring *Wolbachia* strain (wAnga-Mali) was identified in the Malian villages of Dangassa and Kenieroba. Phylogenetic analysis of the nucleotide sequence of two 16S rRNA regions showed that wAnga-Mali clusters with *Wolbachia* strains from supergroup A and has the highest homology to a *Wolbachia* strain isolated from cat fleas (Ctenocephalides). wAnga-Mali is different from Wolbachia strains previously reported in *A. gambiae* from Burkina Faso. Quantitative analysis of *Wolbachia* and *Plasmodium* sporozoite infection in field-collected mosquitoes indicates that the prevalence and intensity of *Plasmodium falciparum* sporozoite infection is significantly lower in *Wolbachia*-infected females. The presence of *Wolbachia* in females from a laboratory *Anopheles coluzzii* (*A. gambiae*, M form) colony experimentally infected with *P. falciparum* (NF54 strain) gametocyte cultures slightly enhanced oocyst infection. However, *Wolbachia* infection significantly reduced the prevalence and intensity of sporozoite infection, as observed in the field. This indicates that wAnga-Mali infection does not limit early stages of *Plasmodium* infection in the mosquito, but rather has a strong deleterious effect on sporozoites and reduces malaria transmission in *An. coluzzii*.

Inhibition of *Aedes aegypti* vector competence by *Wolbachia* is not strictly density dependent

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The introduction of endosymbiotic bacterium *Wolbachia pipientis* from *Drosophila melanogaster* (*w*Mel) into the mosquito vector *Aedes aegypti* restricts transmission of human pathogenic flaviviruses and alphaviruses, including dengue, Zika, and chikungunya. While current field trials utilize *w*Mel-infected *Ae. aegypti* to evaluate the effectiveness of this strategy for disease control, we recently described the generation of 3 new transinfected *Ae. aegypti* lines, carrying *Wolbachia* strains *w*MelCS (*D. melanogaster*), *w*Ri (*D. simulans*) and *w*Pip (*Culex quinquefasciatus*). These strains vary significantly in their effects on host fitness as well as their pathogen blocking activity. Past studies have suggested a correlation between *Wolbachia* strains that reside at high density in a host, and effective pathogen blocking, but what the optimal density is, and in which tissues, is unknown. Here, we provide an examination of the tissue-specific localization and density of *w*Mel, *w*MelCS, *w*Ri and *w*Pip in *Ae. aegypti* and correlate these the ability of each strain to block flaviviruses. We report no trend between *Wolbachia* density in the midgut and restriction of virus infection in the mosquito body, and no trend between *Wolbachia* density in the salivary glands and the ability of DENV to disseminate following an infectious blood meal. Interestingly, *w*Pip, previously shown to restrict the flavivirus West Nile virus (WNV) in its native host, *C. quinquefasciatus*, despite establishing at high levels in *Ae. aegypti* tissues, did not limit DENV or WNV (Kunjin strain) replication following an infection challenge. These results indicate that the pathogen blocking phenotype described for *Wolbachia* is not simply dependent on the concentration of *Wolbachia* in tissues key for viral establishment, but rather seems to be specific to a strain, and/or host-strain interaction. These results provide further insight into the mechanism by which *Wolbachia* restricts viral replication and suggests future line selection should not be based on *Wolbachia* density alone.

A-WOL: A decade of drug discovery and development

Mark Taylor on behalf of the A-WOL consortium

A-WOL aims to discover and develop new drugs against onchocerciasis (river blindness) and lymphatic filariasis (elephantiasis). A-WOL is working to develop novel approaches that are capable of killing adult worms within a 7-day treatment period by targeting *Wolbachia*, a mutualist essential for the worms' survival. The consortium has identified a candidate molecule, TylAMac, which is the first next-generation anti-*Wolbachia* drug to be designed specifically as a macrofilaricidal agent. The lead molecule is undergoing formal preclinical evaluation in partnership with AbbVie. A-WOL has screened the human pharmacopeia, focused anti-infective and large diversity libraries to identify 6 novel anti-*Wolbachia* chemotypes with suitable drug-like qualities. The first industrial scale screening of 1.3 million compounds in partnership with AstraZeneca delivered 20,000 hits and a further 10 novel chemotypes as promising new leads. Several of these lead candidates show rapid killing of *Wolbachia* which can deliver even shorter treatment periods. A-WOL has also identified two repurposing molecules, high-dose rifampicin and fusidic acid, which significantly reduce treatment times compared to the current gold standard, doxycycline. A radical improvement to targeting *Wolbachia* occurs via a drug synergy between a common anthelmintic drug (albendazole) and different classes of antibiotics (tetracyclines and rifampicin). Using the drugs in combination reduced the length of treatment required from several weeks to 7 days, opening up the opportunity to scale-up this approach at the community level.

POSTER SESSION

Evaluation of MinION Nanopore Sequencing for Scaffolding of *Spiroplasma* Genomes and Identifying Prophage Sequences

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Technical innovations such as second-generation sequencing revolutionized bacterial genomic studies by accessing to high-throughput and high-quality sequencing. However, it had been still difficult to obtain complete *de novo* assemblies for bacterial genomes containing repeated DNA elements that are longer than the read lengths achievable with typical short read sequencers. Spiroplasmas are endosymbiotic bacteria associated with a variety of plants and arthropods. Some of them are naturally associated with several *Drosophila* species and cause female-biased sex ratios as a result of selective death of male offspring during embryogenesis. Previous genome sequencing efforts in several *Spiroplasma* species pathogenic to plant or arthropods revealed that their genomes contain highly repetitive phage-related fragments, suggesting that the presence of viral sequences may be a common characteristic of these species. So far, we have been conducting the genome sequencing of a male-killing *Spiroplasma* strain NSRO originated from *Drosophila nebulosa*, its non-male-killing variant NSRO-A, and bacteriophages infecting them. By shotgun sequencing of purified phage genome and direct sequencing of PCR products, we identified single and, at least, three circular phage genomes of 19 kbp for NSRO and NSRO-A, respectively. On the other hand, by whole genome sequencing using a third-generation sequencing system, PacBio RS II, we obtained 10 contigs for each strain. However, terminal sequences of each contig were represented by presumable prophage sequences, which were main obstacles to obtaining the complete chromosomal sequences. For contig scaffolding and identifying prophage sequences, we applied another promising platform, MinION™ nanopore sequencing system. Provided that the DNA strand is kept intact during sample processing, this system has no upper limit to the potential read length. Here, we present attempts for preparing highly intact *Spiroplasma* genome and evaluate the utility of the MinION sequencing system for our aim.

Two-Component Systems in *Wolbachia*: The Loss of Signaling Inputs and Outputs during Host-specific Divergence

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Two-component regulatory systems (TCSs) are commonly used by bacteria to coordinate intracellular responses with environmental cues. These systems are composed of functional protein pairs that include a sensor histidine kinase and cognate response regulator. We used bioinformatic tools to compare predicted TCS relays of streamlined bacterial endosymbiont *Wolbachia pipientis* variants to well-studied, free-living α-proteobacteria, which in some cases carry dozens of these pairs. In comparison to closely related pathogenic *A. phagocytophilum* and *E. chaffeensis* species which harbor three cognate pairs, *Wolbachia* encode only two pairs: CckA/CtrA and PleC/PleD. The genomic context of predicted open-reading frames for TCS components were consistently found clustered with metabolic genes. Although the domain architecture and key functional residues for TCS proteins were well-conserved in *Wolbachia*, residues that specify cognate pairing diverge substantially from other Anaplasmataceae. The loss of the NtrX/NtrY cognate pair, known to regulate metabolic response to environmental nitrogen, suggests that this function was lost prior to divergence of progenitor *Wolbachia* lineages. Further, loss of predicted function associated with the PleC/PleD pair in worm-host associated *Wolbachia* suggests systematic reductions during host association with Supergroups C/D. Overall, these findings indicate that *Wolbachia* retain only core cell-cycle regulatory elements of free-living bacterial systems, insect/nematode-specific reduction, and the potential for cross-talk between cognate TCS pairs.

Transgenic *Anopheles gambiae* expressing the Wolbachia surface protein: immunological effects and implications for malaria control

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There is a strong interest in molecules capable of modulating the immune response of arthropod vectors, in view of their possible applications in the control of vector-borne diseases. The *Wolbachia* surface protein (WSP) from *Wolbachia* of the nematode *Dirofilaria immitis* is recognized to modulate the innate immunity response in humans, mice and mosquito cells. Our aim was to engineer *Anopheles gambiae* mosquitoes, in order to obtain WSP-producing transgenic lines, with protein expression under the control of the blood-inducible vitellogenin promoter, for a specific production of WSP following the blood meal. First, we investigated the production of the protein, the stimulation of immune responses induced by WSP expression in female mosquitoes, and the fitness of these transgenic insects. Second, we tested the capacity of these mosquitoes to interfere with the infection by *Plasmodium*. One of the WSP-producing transgenic lines determined the activation of genes of the immune system and a strong reduction of the parasites burden after the infection. In conclusion, this work presents the engineering of a mosquito for the production of a protein from a symbiont, opening the way toward further studies related with insect immunity and *Wolbachia*, and provides evidence that WSP-induced immune activation could interfere with malaria transmission by mosquitoes.

Understanding *Wolbachia* invasions using two hybridizing *Drosophila* species

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A major question in the field of *Wolbachia* research is what are the conditions under which a *Wolbachia* infection spreads through and then is maintained in a host population. Here we study *Wolbachia* infection dynamics in two closely related species of *Drosophila* flies that are sympatric and hybridize in nature. One of them, *D. recens*, harbors a *Wolbachia* infection (wRec) at high prevalence in natural populations, while the other species, *D. subquinaria*, has never been found to harbor *Wolbachia* in natural populations. About 3% of wild-caught *D. subquinaria* harbor a *D. recens* mtDNA haplotype, but none of these flies also harbor *Wolbachia,* indicating the introgression and subsequent loss of *Wolbachia* infection after hybridization. In *D. recens*, *Wolbachia* causes cytoplasmic incompatibility (CI), with nearly complete embryo mortality in the incompatible cross. Conversely, *Wolbachia*-infected *D. subquinaria* generated via laboratory introgressions have been reported to show male killing in some host genetic backgrounds and no evidence for CI. We introgressed wRec into recently collected lines of *D. subquinaria* and found very strong CI in this host, in fact CI is stronger than in *D. recens*. Furthermore, we have not recovered the male-killing phenotype in any recently collected host strain. Recent research suggests that some CI-causing *Wolbachia* strains have the unique ability to invade many novel host species on a relatively short time scale. What inhibits the invasion of wRec into *D. subquinaria*? To assess this, we predict theoretical equilibrium infection frequencies for *D. recens* and *D. subquinaria* based on (1) presence and/or strength of reproductive manipulations in both species, (2) maternal transmission rates, and (3) relative fecundity of *Wolbachia* infected females. We then use models of *Wolbachia* frequency dynamics to ask whether these models are sufficient to explain empirical infection frequencies in nature.

The *Wolbachia*-quill mite-bird system: model for exploring endosymbiont diversity, transmission and evolution

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Quill mites (Acariformes: Syringophilidae) are permanent, mono- and stenoxenous bird ectoparasites living inside the quills of feathers. For several reasons, quill mites represent unique *Wolbachia* hosts: *(i)* Their tight associations with bird host enable investigating the spread of the bacterium and co-phylogenetic patterns. *(ii)* Their diet: consists of bird host subcutaneous tissue (lymph, blood) and may necessitate on bacterial suplementation. Furthermore, bird host fluids may facilitate bacterial transmission between quill mite species. (*iii*) Their populations are often strongly female-biased, suggesting a potential reproductive effect of *Wolbachia*.

Our previous studies on the syringophilid-*Wolbachia* associations (based on the genes *ftsZ*, *gltA*, *groEL*, *coxA* and the16S rRNA) revealed unusually high bacterial diversity (three supergroups including two earlier unknown) in 11 individuals of 5 quill mite species and hinted at both mite phylogeny and bird hosts as potential determinants of *Wolbachia* distribution. Here, we used 16S rRNA amplicon sequencing of various species of quill mites to determine other bacterial taxa that may be important in their biology. Our results confirm the previous assumption that *Wolbachia* is a common symbiont of quill mites. Additionally, we observed that the abundance of *Wolbachia* differed significantly across developmental stages increasing from the larvae through nymphs to adults. We further detected *Spiroplasma* symbionts in three quill mite species that share common bird hosts, suggesting that these bacteria may have been exchanged via the bird host. Our study not only broadens the knowledge of symbiont diversity in quill mites but also raises questions on the role of these bacteria in quill mite biology.

New scenario of the *Wolbachia*-induced feminization of males of the crustacean isopod *Armadillidium vulgare*

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In male crustaceans, the androgenic glands synthesize the androgenic gland hormone (AGH) which is responsible for the differentiation of primary and secondary male sexual characters. In terrestrial isopods, this gland does not develop in male embryos that have inherited the feminizing α-proteobacteria *Wolbachia*. These genetic males rather differentiate into functional females inducing a female-biased sex ratio in the population, that favor the spread of the bacteria. Several intersex individuals appear in these populations, resulting from incomplete feminization of genetic males. Primary and secondary female sex characters, including genital apertures, may appear in these individuals. *Wolbachia* can also induce hypertrophy of the androgenic glands in intersex males which usually leads to sterile individuals. We have investigated short term and long term effects of *Wolbachia* on *Armadillidium vulgare* sexual phenotype. We thus showed that the *Wolbachia*-induced intersex phenotypes depend on the bacterial load. Using RNA silencing, we also showed that *Wolbachia* could induce a dysfunction of the biological pathway of the androgenic hormone. These results allowed establishing a new scenario of the action mode of *Wolbachia* whose effect would be potentiated by AGH, and open new perspectives to analyze gonad differentiation mechanisms and how feminizing endosymbionts interfere with this process.

Wolbachia-induced apoptosis associated with increased fecundity in *Laodelphax striatellus* (Hemiptera: Delphacidae)

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Wolbachia influence the fitness of their invertebrate hosts. Although *Wolbachia* effects on reproductive incompatibility are well characterized, *Wolbachia* also influence egg production but the mechanistic basis of fecundity effects is unclear. Here, we investigate whether apoptosis, which has been implicated in fecundity in model insects, influences the interaction between fecundity and *Wolbachia* in the planthopper, *Laodelphax striatellus*. *Wolbachia*-infected females produced about 30% more eggs than uninfected females. We used TUNEL staining to visualize apoptosis. Microscopic observations indicated that the *Wolbachia* strain *w*Stri increased the number of ovarioles that contained apoptotic nurse cells in both young and aged adult females. The frequency of apoptosis was much higher in the infected females. The increased fecundity appeared to be due to apoptosis of nurse cells, which provides nutrients to the growing oocytes. In addition, cell apoptosis inhibition by caspase mRNA interference (RNAi) in *Wolbachia* infected *L. striatellus* markedly decreased egg numbers. Together, these data suggest that *w*Stri might enhance fecundity by increasing the number of apoptotic cells in the ovaries in a caspase-dependent manner. Our findings establish a link between *Wolbachia*-induced apoptosis and egg production effects mediated by *Wolbachia,* although the way that the endosymbiont influences caspase levels remains to be determined.

The paternal mother effect on penetrance of *Wolbachia*-induced cytoplasmic incompatibility

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Field and laboratory studies report inconstant degrees of embryonic lethality caused by *w*Mel-induced cytoplasmic incompatibility (CI) in *Drosophila melanogaster*. Several factors can influence CI penetrance including *Wolbachia* titer and CI gene expression, as well as host genotype, age, mating rate, rearing density, rearing temperature, and rate of male development. Despite strict control of these variables, low penetrance of CI can still manifest. Here, we report a transgenerational influence of mother age on the penetrance of CI induced by her sons. Specifically, older mothers (11 days of age) yield sons that induce stronger CI as compared to younger mothers (2 and 5 days of age), a relationship we term the 'Paternal Mother Effect' (PME). Modulation of paternal mother age did not influence the ability of CI to be rescued. We test three hypotheses related to the PME: as a mother ages i) her *Wolbachia* titers increase, and the titer transfer to her sons likewise increases, ii) her *Wolbachia* titers remain constant but her sons' titers increase, and iii) expression of the CI genes, *cifA* and *cifB*, increases in her sons. Our results support a transgenerational influence of mother age on CI penetrance and have implications for both field and laboratory studies where precise control over levels of *w*Mel-induced CI will be valuable for dissecting the genetic and functional basis of CI.

Localization and dynamics of *Wolbachia* infection in Asian citrus psyllid *Diaphorina citri*, the insect vector of the causal pathogens of Huanglongbing

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Abstract: *Wolbachia* is a group of intracellular bacteria that infect a wide range of arthropods including the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama. This insect is the vector of *Candidatus* Liberibacter asiaticus (CLas), the causal pathogen of Huanglongbing or citrus greening disease. Here, we investigated the localization pattern and infection dynamics of *Wolbachia* in different developmental stages of ACP. Results revealed that all developmental stages of ACP including egg, 1st–5th instar nymphs, and adults of both gender were infected with *Wolbachia*. FISH visualization of an ACP egg showed that *Wolbachia* moved from the egg stalk of newly laid eggs to a randomly distributed pattern throughout the egg prior to hatching. The infection rate varied between nymphal instars. The titers of *Wolbachia* in fourth and fifth instar nymphs were significantly higher than those in the first and second instar nymphs. *Wolbachia* were scattered in all nymphal stages, but with highest intensity in the U-shaped bacteriome located in the abdomen of the nymph. *Wolbachia* was confined to two symmetrical organizations in the abdomen of newly emerged female and male adults. The potential mechanisms of *Wolbachia* infection dynamics are discussed.

Genome Sequencing and Analysis of the *Wolbachia* Endosymbiont of *Brugia pahangi*

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Wolbachia is an intracellular endosymbiont of many filarial parasites, including the agents of neglected tropical diseases such as onchocerciasis and lymphatic filariasis. The obligate nature of this symbiosis has been successfully leveraged to identify new drug targets for filariasis. Here we present the genome sequence and analysis of the *Wolbachia* symbiont of *Brugia pahangi* (wBp), a closely related species of the human filarial nematode *B. malayi.* The wBp and wBm are 99.30% identical sharing 1.05 Mbp, corresponding to 98.18% of the 1.07 Mbp wBp genome and to 97.52% of the 1.08 Mbp wBm genome. Shared and unique genes between the two strains were identified, as well as pseudogenes and genome rearrangements. Despite the high nucleotide sequence identity, the genome has nineteen breaks in synteny with nine inversions. Due to the close interdependence between *Wolbachia* and their filarial hosts, the symbionts` genomes are likely to be shaped by the worm`s biology, infection mechanisms and selective pressures. Therefore, genomic comparisons between *Wolbachia* from different filarial nematodes can provide information useful to improve our understanding of the *Wolbachia*-nematode symbiosis.

Transfection of buffalo flies (*Haematobia exigua*) with *Wolbachia*

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Buffalo flies (BF), *Haematobia exigua,* are obligate hematophagous parasites of pastured cattle responsible for significant production losses and animal welfare impacts in northern Australian cattle industries. They are exotic pests that have been steadily increasing their range southward under the effects of climate change. Control of BF currently relies primarily on the use of insecticides but issues with resistance and consumer preference for commodities produced in low chemical systems have increased pressure for the development of new means of control.
The endosymbiotic bacterium *Wolbachia* can have wide ranging effects on host biology including the induction of cytoplasmic incompatibility, modification of host fitness and impacts on vector competence. These properties have made it of intense interest for use in novel control approaches for a range of insect pests. As part of a program to investigate the potential for use of *Wolbachia* in area wide control of BF we have surveyed Australian BF populations and found that *Wolbachia* is not present. Thus the first step in assessing the effects of *Wolbachia* is the establishment of stably transinfected strains of BF. Here we describe the tissue distribution profiles and infection dynamics of three different *Wolbachia* strains (*w*AlbB, *w*Mel and *w*MelPop) in transinfected BF and report the effects of transinfection on insect longevity.

A Novel Tool for Detecting Lateral Gene Transfer – LGTSeek

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While abundant, lateral gene transfers (LGTs) between endosymbionts and their hosts remain difficult to detect. Partially this is due to the presence of reads from the endosymbiont genome in the raw sequencing data used in the host assembly. But additionally, there are multiple copies of the same or similar DNA integrations that collapse in assemblies, requiring assembly-free read-based algorithms that specifically detect junctions between donor DNA and recipient DNA. Here, we present LGTSeek, a novel tool for detecting LGTs in hosts based on paired end reads. LGTSeek identifies putative junction spanning pair of reads (JSPRs) indicative of potential LGTs by differential mapping to one or more reference genomes to identify read pairs where one read maps to the host while the other maps to the donor. These putative JSPRs are subsequently searched against the NCBI nucleotide database with BLAST and filtered based on hits to the desired taxa. As a proof of concept, publicly available genome sequencing data on the western corn rootworm (*Diabrotica virgifera*) generated by Monsanto were analyzed with our tool for putative LGTs between its bacterial endosymbiont *Wolbachia* and the host rootworm. An average of 82 reads were identified per sample that strongly support the presence of at least one LGT within *Diabrotica virgifera*, with paired end reads that have top hits to related species to *Diabrotica* and *Wolbachia*. Overall, this example showcases a novel tool for identifying LGTs over a wide range of taxa for species.

Bare Necessities: Investigating the Requirements for Establishing a *Wolbachia* Infection

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The intracellular bacterium *Wolbachia pipientis* is currently being explored as a potential tool to control the spread of arthropod-borne diseases due to its ability to inhibit the replication of certain RNA viruses. If this method is to be adopted more widely, *Wolbachia* must be artificially transferred into insect species with which it has not already formed an association. Since colonization is the first step in the creation of this tool, our research aims to elucidate the requirements for the relationship between *Wolbachia* and its host. We are exploring host genomic regions important for the establishment of this infection. To do this, we obtained fruit fly lines that contain chromosomal deficiencies maintained in a balanced, hemizygous state to screen the second and third chromosomes. As these lines were generated in a *Wolbachia*-infected background, we first identified 42 fruit fly stocks that had lost their *Wolbachia* infection. To then determine which of these regions are necessary for *Wolbachia* to establish an infection, we attempted to re-infect these lines. Of those 42 lines that had lost the *Wolbachia* infection, only 4 were unable to reacquire it. One of these regions has been narrowed down to two potential genes of interest. We additionally asked if these host genetic regions are important for the establishment of any endosymbiotic infection by attempting to infect these same 4 lines with the facultative, male-killing bacterium *Spiroplasma poulsonii*. Infection was successful, therefore these 4 regions are important for the establishment of a *Wolbachia* infection, but not a *Spiroplasma* infection. To locate all regions important for the establishment of a *Wolbachia* infection, we will screen the remaining 2 chromosomes in the same manner.

Impact of the ecological and endosymbiotic status on gut bacteria diversity in wild *Drosophila melanogaster* larvae

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The vast majority of multicellular organisms are naturally colonized by multiple microbial taxa that together constitute the microbiota. Bacteria represent a large proportion of the microbiota, being both horizontally and vertically transmitted. In *Drosophila*, gut bacteria are environmentally acquired from the diet whereas the endosymbiotic bacterium *Wolbachia* is inherited from the previous generation. However, the impact of the presence of *Wolbachia* on the diversity and/or the density of gut bacteria in the field is still unknown. We addressed this question using wild *Drosophila* larvae, collected on different fruit baits in two field stations in France. Approximately 220 *Drosophila*-like larvae were collected and individual guts were dissected. We developed a PCR-RFLP assay to accurately and rapidly identify *Drosophila melanogaster* species among all the collected larvae. *Wolbachia* prevalence and density were determined by qPCR for each larvae, in all experimental conditions. A pilot experiment revealed for the first time the gut bacterial diversity of wild, *Wolbachia* infected and *Wolbachia*-free larvae. Using this experimental set-up, we tried to address the importance of the genotype, the environment as well as the endosymbiont status on gut bacterial diversity in insects using the model organism *Drosophila*.

Wolbachia restores normal oogenesis in *Drosophila* hosts lacking factors for germ cell maintenance and differentiation

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Wolbachia infections have been shown to rescue null mutations in sex-lethal (*sxl*) and bag-of-marbles (*bam*) genes, both of which are required for maintenance of the germ cell niche and normal oocyte differentiation. Recently, a *Wolbachia* gene involved in *sxl* rescue, *TomO*, was identified and characterized. However, this gene alone does not fully recapitulate the *sxl* rescue phenotype, indicating that there are more factors involved. Here, we present data showing that *Wolbachia* is capable of rescuing another critical host factor involved in early oogenesis, *meiotic P26* (*mei-P26*). In uninfected *Drosophila melanogaster* bearing homozygous null mutations or expressing RNAi constructs for *mei-P26*, 69% of oocytes develop with tumorous phenotypes due to the overproliferation of nurse cells. However, when these genotypes were infected with *Wolbachia* by crossing to infected females, the significantly more oocytes developed normally, with only 18% exhibiting nurse cell over-proliferation. Interestingly, this gene was also a positive hit in a previously published cell culture RNAi screen for genes that reduce *Wolbachia* titer. Considering that *mei-P26* is also a ubiquitin ligase, and *Wolbachia* is thought to use byproducts of host proteolysis for nutrition, this gene may play dual roles in the association. Ongoing work seeks to fully understand the role of Mei-P26 in *Wolbachia* transmission and titer.

Functionally attuned symbionts of turtle ants - stage-specific gut bacteria recycle nitrogen and scavenge carbon

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Ants are among the most abundant groups of animals in terrestrial environments. Surprisingly, only a subset harbor high density communities of symbiotic gut bacteria, setting them apart from many animals. We know little about the functions of such symbionts in ants, even in symbiotic "hotspot" lineages, where specialized bacteria have colonized host guts for millions of years. Turtle ants of the genus *Cephalotes* hold promise in addressing these mysteries. Like a number of ants harboring large density gut communities, their diets are generally poor in nitrogen. And further like these other hotspot hosts, turtle ant *workers* harbor a stereotyped suite of behaviorally transmitted, specialized gut bacteria. In this study we use both experimental and (meta)genomic tools, to dissect the functions of these ancient gut symbionts. We find that several symbionts of turtle ants play specialized roles in nitrogen recycling, and that nearly all gut symbionts can assimilate recycled nitrogen, using it to make all or most amino acids. *Workers* acquire symbiont-produced amino acids in large quantities, and the conserved genomic signatures across symbionts from 17 turtle ant species suggest an ancient capacity for symbiont-mediated exploitation of nitrogen poor diets. In contrast to these discoveries, the first multi-species survey of *larvae*-associated bacteria in turtle ants, shows that some symbionts are highly related to free-living bacteria, and either rare or absent in *workers*. Complementing this taxonomic divergence between stages, our metagenomic analyses suggest functional divergence, as well. Perhaps of greatest interest are the capacities for *larvae*-specific bacteria to scavenge carbon from pectin, a recalcitrant carbohydrate abundant in the pollen-rich diets of turtle ants. These findings combine to raise the possibility of a developmentally partitioned symbiosis, in which *larvae*-associated symbionts derive usable carbon, aiding *workers'* energy budgets, while symbionts in *workers* produce essential forms of nitrogen, of potential use for developing *larvae*.

Wolbachia infection levels in natural populations of *Drosophila melanogaster* under ambient radiation in Ukraine

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It is known that endosymbiotic bacteria *Wolbachia* are highly widespread in Cuticulata, in particular in wild populations of *Drosophila melanogaster*. Endosymbionts' prevalence depends on several reproductive alterations in arthropods which allow their spread in host populations [Weinert et al. 2015]. One of the most conspicuous abiotic factors, that has a wide range of impacts on organism's fitness and genetic variability, is ionizing radiation. Even though there is no relationship between radiation resistance and *Wolbachia* in laboratory lines of fruit fly [Vaisnav et al. 2014], nothing is known about the effect of *Wolbachia* infection on the nature populations of *D. melanogaster* under environmental radiation background.

The purpose of our study was to survey relationship between the level of infection with *Wolbachia* in *D. melanogaster* and ambient radiation from different Ukrainian environments: clean localities – 0.07-0.16 μSv/h (Varva, Kyiv, Uman) and contaminated localities – 0.25-13.5 μSv/h (NPP Cooling Pond, Chernobyl, Poliske, Yaniv, Kopachi – Chernobyl exclusion zone). We analyzed 359 isofemale lines for the presence of *Wolbachia* infection by PCR method. The measuring of ambient radiation levels was performed in 2014-2015. All fruit fly populations were obtained in August-September 2014-2015.

We did not observe any correlation between ambient radiation level and the level of Wolbachia infection in 2014 (Spearman rank correlation: r_s = - 0.714, n = 7, p = 0,08) neither in 2015 (r_s = -0.205, n = 5, p = 0.74) nor in 2014-2015 (r_s = -0.5639, n = 12, p = 0.056).

Complete circular genome sequence of the *Wolbachia w*AlbB endosymbiont of *Aedes albopictus*

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Wolbachia is an intracellular alpha-proteobacteria closely related to *Rickettsia*. It is a maternally transmitted, intracellular symbiont of arthropods and nematodes and estimated to infect 40-60% of arthropod species. The tiger mosquito *Aedes albopictus* is naturally infected with *Wolbachia* strains *w*AlbA and *w*AlbB. The cell line Aa23 established from *Ae. albopictus* embryos retains only the *w*AlbB strain and is used as a key model to study host-endosymbiont interactions. The available *w*AlbB genome with 49 scaffolds encompassing 165 contigs is incomplete, hampering a comprehensive analysis of the genome. We have assembled the complete circular genome of a *w*AlbB strain from the Aa23 cell line using long-read PacBio sequencing data at 400X coverage. The assembled circular chromosome is 1.48 megabases in size, an increase of more than 300 kb over the published *w*AlbB genome, making it one of the largest sequenced *Wolbachia* genomes to date. The annotation of the genome identified 1,418 protein-coding genes, 34 tRNA, 3 rRNA and 1 tmRNA loci. The long reads enabled sequencing over complex repeat regions which have been difficult to resolve with short-read sequencing. The availability of a complete circular genome from *w*AlbB will enable further biochemical, molecular and genetic analyses on this strain and related *Wolbachia*.

Evidence for the natural occurrence of *Wolbachia* in *Aedes aegypti*

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Wolbachia are maternally inherited bacteria, infecting a wide range of arthropods and a few nematodes through parasitic or mutualistic affiliation. In addition, it induces reproductive abnormalities in the host, besides reducing their life span. Interestingly such infections confer protection for their host against invading pathogens. Even though *Wolbachia* inhabits several mosquito species, their association is seldom found in disease transmitting vectors. A remarkable example is the major arboviral vector *Aedes aegypti* in which *Wolbachia* infection is hardly reported. In the present study, for the first time, we report the evidence of natural *Wolbachia* existence in *Ae. aegypti*, collected from Coimbatore, India. PCR amplification of *Ae. aegypti* was done with *Wolbachia* specific 16S rRNA, *wsp* and MLST gene primers. BLAST search and phylogenetic tree of the concatenated gene sequences yielded foolproof evidence on the occurrence of *Wolbachia* strains, which affiliates to supergroup B of *Ae. aegypti*. The complete absence of *Wolbachia* in tetracycline treated mosquitoes over generations, evidenced by transmission electron microscopy further adds credence to our observations that the field collected *Ae. aegypti* were indeed infected with *Wolbachia*, which are designated as *w*Aeg. These mosquitoes, no doubt, would be expected to play an important role in arresting multiplication of mosquitoes and thereby impinching transmission of dengue disease. Extensive randomized field trails may be necessary before any logical conclusion would be drawn on the effectiveness of *w*Aeg mosquitoes in inducing reproductive abnormalities besides impeding disease transmissions.

Variation in *Wolbachia* effects on *Aedes aegypti* through the lens of dose-dependent, dynamic mathematical models

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Although *Wolbachia* seems to confer protection against viral infection in a series of invertebrate hosts — like disease-transmitting mosquitoes — in some cases it has been shown to have no effect, or even increase susceptibility. Also, the degree of protection — when present — does vary with host and symbiont species and genetic background, as well as environmental factors, which are often overlooked in lab experiments. External factors like the concentration of the viral inoculum is a determinant of infection success, and may be very important in establishing whether *Wolbachia* protects the host in all conditions, or at least in those to which they are exposed in the field, or not. Dose-response models are required to estimate the mean effect from this kind of data, and the variance of the effect is also an important parameter that can affect the global effect of the symbiont in the field. In this work we show a dose-response model that incorporates heterogeneous probability of infection through an underlying distribution can be used to estimate the mean and variance of host susceptibility. In both data in the literature, — from Vietnamese *Aedes aegypti* — as well as our data — from Brazilian mosquitoes carrying *w*Mel — the symbiont is inferred to increase mean susceptibility, as well as the variance of the effect. This model allows not only a quantitative inference of biological heterogeneity, but it also explains a feature observed across the different experiments: *Wolbachia* seems to increase susceptibility at low doses, but protects at higher doses. The consequences of these estimates are explored using a mathematical model of dengue transmission in the population, and beyond the dose-response model a closer look at the infection data is taken by looking at the levels of infection along time for the different doses.

Maternally inherited, non-bacterial male killer in *Ostrinia scapulalis*: searching for a novel male-killing factor

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Maternally transmitted male killing is widely observed in arthropods. Most of them are caused by bacterial endosymbionts of diverse taxa. We focus on non-bacterial male killer(s) found in a moth, *Ostrinia scapulalis* (Lepidoptera; Crambidae). Cytogenetic observation revealed, in three matrilines, that males died during larval development. RT-PCR showed that a sex-determining gene *Osdsx* (*doublesex* ortholog in *O. scapulalis*) in cytogenetically male hatchlings, which are destined to die, exhibited female-specific splicing. Furthermore, expression levels of Z chromosome-linked genes were abnormally high in male individuals. These results suggested that these male-killing matrilines have the same or very similar phenotype with *Wolbachia*-infected matrilines in *O. scapulalis*, wherein improper dosage compensation is associated with the death of cytogenetic males that were determined as female. Ribosomal RNA-depleted RNA-Seq revealed that a transposon-like sequence was found only in these particular matrilines. We are trying to reveal whether the transposon-like sequence is responsible for the male-killing phenotype.

Some don't like it hot: A temperature increase can be more effective than doxycycline at depleting *w*MelPop-CLA from RML-12 mosquito cells

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There is considerable interest in the resilience of *Wolbachia* and other symbionts to stress conditions experienced or imposed by their invertebrate hosts, from physical extremes (*e.g*., fluctuations in temperature, humidity and day-length), to chemical stressors (*e.g*., insecticides, environmental toxins and antibiotics), and biological challenges (*e.g*., nutrient deprivation, co-infecting organisms, and immune responses). For instance, natural and artificial *Wolbachia* infections in mosquitoes may differ in temperature susceptibility; while in filarial nematodes, *Wolbachia* is known to tolerate prolonged antibiotic chemotherapy and can recrudesce if treatment duration is insufficient. These phenomena have implications for *Wolbachia*-based control methods dependent on either the spread of *Wolbachia* in vectors, or the elimination of *Wolbachia* from filarial worms. Here, we show by qPCR and qRT-PCR that *w*MelPop-CLA infection in the *Aedes albopictus* cell line RML-12 can be depleted by doxycycline to almost undetectable levels within four weeks at 28°C. When the incubation temperature is increased to 34°C, *w*MelPop-CLA in the absence of antibiotics is apparently eliminated within three weeks. However, if doxycycline is added to RML-12 cultures maintained at 34°C, *w*MelPop-CLA is cleared in the same timeframe with identical kinetics. Thus, even with weekly subculture, the rate of depletion of the symbiont reaches a plateau when two stressors are applied simultaneously. We found no evidence for gross effects on host cell viability at elevated temperatures, suggesting that the susceptibility phenotype is intrinsic to the symbiont. This experiment raises important questions regarding the regulation of stress responses in *Wolbachia* and specifically, if it exhibits a "one-size-fits-all" mechanism in the face of fundamentally different types of stress. Moreover, the differential survival of various *Wolbachia* strains at elevated temperatures, including the ability of filarial *Wolbachia* to thrive in endothermic hosts, demands further investigation.

Targeting *Wolbachia* through synergistic drug combinations

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Elephantiasis and African River Blindness are human diseases caused by filarial nematodes. These organisms afflict millions globally and there are currently no drugs that efficiently target the long-lived adult nematodes. These nematodes exist in a symbiotic relationship with the intracellular bacteria Wolbachia. Strikingly Wolbachia is required for both nematode fertility and survival. Thus directly targeting Wolbachia serves as a promising alternative approach to combating filarial nematode mediated diseases. Taking advantage of high throughput cell-based screens and fluorescent based secondary screens in adult nematodes, we have identified novel compounds that exhibit potent anti-Wolbachia properties. Using adult nematodes, Wolbachia-infected Drosophila cell lines and oocytes, we will present our results assaying combinations of known and novel compounds for synergistic anti-Wolbachia activity.

Impact of *Wolbachia* on viral life cycle in mosquito cells

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In the absence of effective drugs and vaccines, a novel dengue/Zika control strategy is being developed to utilize the endosymbiotic bacterium *Wolbachia* for reducing the ability of mosquitoes to transmit disease. Although *Wolbachia*is known to inhibit dengue virus (DENV) and Zika virus (ZIKV) in mosquitoes, it is unclear how it interferes with each stage of the viral life cycle. In this study, we have carried out the DENV and ZIKV binding assay to investigate the impact of *Wolbachiaw*AlbB on viral attachment. The amount of DENV negative strand RNA was also measured and compared between *w*AlbB-infected and -uninfected *Aedesaegypti* Aag2 cells to define the impact of *Wolbachia* on viral replication. Our results showed that, in addition to suppression of viral replication, *Wolbachia*-induced reduction in viral binding to Aag2 cells. These results will aid in understanding the mechanism of *Wolbachia*-induced pathogen resistance and developing novel control strategies for mosquito-borne diseases.

A gut filling: the kinetics of *Wolbachia* infection of Drosophila digestive track

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There is no consensus on why *Wolbachia* bacteria colonize specific cell types in host tissues. We are addressing this question in the Drosophila gut. We have recently shown that *Wolbachia* infect the gut epithelium and modulates the microbiome composition of infected fruit flies (Simhadri *et al.* 2017). Infection patterns in *Drosophila melanogaster* digestive tracts show *Wolbachia* homogeneously infect all regions in larval guts, but colonize adult guts in a very distinct way. In adult foreguts and midguts, isolated patches of infected cells are observed, while the hindgut is infected in its totality. These results were reproducible in flies with different genetic backgrounds, infected with two different strains of *Wolbachia: w*Mel and *w*Mel Pop. Our hypothesis is that the different patterns of infection reflect the *Wolbachia* infection status of progenitor stem cells, suggesting that Wolbachia are only transmitted through the gut cell lineage, with no occurrence of cell-to-cell transmission. We believe that in larvae and hindgut, all progenitor cells are infected, thus the whole tissue becomes infected. For adult intestinal stem cells (ISC), on the other hand, only specific progenitor stem cells are infected are infected. We are currently testing this hypothesis, with lineage labelling and markers for the various cell types in the gut. These experiments will provide insights into the underlying mechanisms of *Wolbachia* infection into different cells in a given tissue. Furthermore, it will allow us to assess whether the presence of *Wolbachia* in a progenitor cell affects the intestinal stem cell division rate and patterns of stem cell differentiation.

Simhadri, R. K., et al. (2017). "The Gut Commensal Microbiome of *Drosophila melanogaster* Is Modified by the Endosymbiont *Wolbachia*." mSphere **2**(5).

Multiplexed Complete Microbial Genomes on the Sequel System

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Microbes play an important role in nearly every part of our world, as they affect human health, our environment, agriculture, and aid in waste management. Complete closed genome sequences, which have become the gold standard with PacBio longread sequencing, can be key to understanding microbial functional characteristics. However, input requirements, consumables costs, and the labor required to prepare and sequence a microbial genome have in the past put PacBio sequencing out of reach for some larger projects. We have developed a multiplexed library prep approach that is simple, fast, and cost-effective, and can produce 4 to 16 closed bacterial genomes from one Sequel SMRT Cell. Additionally, we are introducing a streamlined analysis pipeline for processing multiplexed genome sequence data through de novo HGAP assembly, making the entire process easy for lab personnel to perform. Here we present the entire workflow from shearing through assembly, with times for each step. We show HGAP assembly results with single or very few contigs from bacteria from different size genomes, sequenced without or with size selection.

Wnt Signaling regulates *Wolbachia* in the *Drosophila* Germline via Proteolysis

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Being maternally transmitted, *Wolbachia* bacteria are under selective pressure to infect the female germline. Current work in our lab demonstrates that Wnt signaling is an important regulator of *Wolbachia* tropism to the germline of the fruit fly *Drosophila melanogaster*. Wnt signaling is highly conserved throughout animal evolution and is a key regulator of embryonic development and cell proliferation. However, the mechanism of regulation of *Wolbachia* tropism by Wnt signaling remains to be elucidated. It has been previously shown that *Wolbachia* relies on host amino acids that are made available by proteolysis for survival and growth (1, 2). Here, we propose that Wnt signaling in the germline upregulates proteolysis, which subsequently regulates the density of *Wolbachia w*Mel. Upregulation of Wnt signaling in the female germline with a constitutively active β-catenin mutant, the major positive regulator of the Wnt pathway, increases proteasome activity, as measured by biochemical assays using fluorogenic substrates. This study indicates proteolysis to be a link between Wnt signaling and *Wolbachia* tropism in the *Drosophila* gonads. These finding help us further understand the complex interactions between *Wolbachia* and their host intracellular environment.

References:

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Impact of *Wolbachia* on transmission of tomato spotted wilt virus by thrips

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As the most successful endosymbiotic bacteria in the world, *Wolbachia* infect up to 65% insect species in nature and interact with host physiological systems in a variety of ways, resulting in alteration of their reproduction, immunity or metabolism. Both the western flower thrips, *Frankliniella occidentalis,* and its relative species *F. intonsa,* are vectors of tomato spotted wilt virus (TSWV), a negative-sense single-stranded RNA virus, which causes serious diseases of many economically important plants worldwide. We found a high infection rate of *Wolbachia* in *F. intonsa* natural population, in contrast to absence of *Wolbachia* in *F. occidentalis* population. This infection pattern is coincidence with a lower vector competence of *F. intonsa* for TSWV as compared to *F. occidentalis,* indicating potential role of *Wolbachia* in viral interference in thrips. We will discuss our results in relation to interaction of *Wolbachia* with thrip's immune and metabolic pathways and the potential implementation of *Wolbachia* in controlling TSWV transmission.

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